COURTESY COPY OF THE PCT APPLICATION AS ORIGINALLY FILED WITH ABSTRACT

Quasirandom structure and function guided synthesis methods

Technical Field of the Inv ntion

One aspect of the present invention is directed to methods for the synthesis of molecules in which the steps of synthesising the molecule from a plurality of reactants or functional entities is guided by connector polynucleotides (CPNs) capable of hybridizing to complementary connector polynucleotides (CCPNs) harbouring at least one functional entity comprising at least one reactive group.

As at least one of said CCPNs hybridize to at least two CPNs, it will be possible to bring together at least two CPNs to which further CCPNs can hybridize. Accordingly, each CPN will "call" for one or more CCPNs capable of hybridising to the CPN.

Following the formation in the above-described way of a supramolecular hybridization complex comprising a plurality of CPNs and a plurality of CCPNs, the reaction of reactants or functional entity reactive groups result in the formation of at least one molecule comprising the reaction product generated by the reacted reactants, such as e.g. a molecule comprising covalently linked functional entities.

The formation of the molecule involves reacting the plurality of reactants, said reactions resulting e.g. in the transfer of functional entities from one or more "donor CCPNs" to at least one "acceptor CCPN" with which the transferred functional entities were not covalently associated prior to the transfer.

Transfering at least one functional entity from one CCPN to another CCPN and reacting the reactants can in one embodiment result in the formation of a molecule e.g. comprising covalently linked functional entities without the "donor CCPNs" being covalently linked once the molecule has been generated. Accordingly, in one embodiment, once the reactants have reacted and the molecule has been formed, the "donor CCPN's" are not covalently linked e.g. by covalent bonds between functional entities constituting the molecule. In this embodiment, the cleavage of covalent bonds between reactants, or functional entities, and "donor CCPNs", also prevent the "donor CCPNs" from being covalently linked to each other.

Both the CPNs and the CCPNs comprise a polynucleotide part. The formation of the molecule comprising reacted reactants, such as e.g. covalently linked functional entities, does not involve a step of cleaving the polynucleotide part of a CPN or a CCPN. In this way the methods of the present invention are different from state of the art polynucleotide ligation and restriction reactions.

Background of the Invention

Ribosome mediated translation involves hybridising the anti-codon of tRNAs to a mRNA template and generating a bond between the amino acid residues harboured by the tRNAs. Only 2 reactive groups are reacted in order to generate the peptide bond between neighbouring amino acid residues in the growing peptide chain. Ribosome mediated translation employs the principle of template directed synthesis and does not involve hybridization of a plurality of connector polynucleotides (CPNs) to a plurality of complementary connector polynucleotides (CCPNs). Another difference between ribosome mediated translation and the method of the present invention is that in the present method for synthesising at least one molecule, at least 1 CCPN hybridizes to at least 2 CPNs.

Additional examples of template directed synthesis methods are disclosed in WO 93/03172 (Gold et al.) and WO 02/074929 (Liu et al.). The methods of the present invention are not related to template directed synthesis as no templates are employed in the methods of the present invention.

Enzymatic ligation and chemical ligation are processes well known in the art. In some cases only 2 reactive groups react in order to generate a product. An example is a reaction between e.g. a 5'-phosphate group of a nucleotide and a 3'-hydroxy group of another nucleotide.

In one embodiment of the present invention, the synthesis and formation of a molecule in accordance with the methods of the present invention does not result in polynucleotides being covalently linked once the molecule has been formed. Rather, the plurality of CCPNs having donated functional entities to the synthesis of the molecule comprising reacted reactants, such as e.g. covalently linked functional entities, remain hybridised to one or more CPNs and do not become covalently linked

once the molecule comprising covalently linked functional entities has been generated.

Summary of the Invention

The present invention alleviates a number of short-comings associated with prior art methods and solves a number of problems related to the limited applicability of template directed synthesis methods used for generating large libraries of molecules.

Template directed synthesis employs a single template of covalently linked nucleotides for the synthesis of a molecule. Once the template is defined by its sequence the number and kind of anti-codons or transfer units capable of hybridizing to the codons of the template have de facto also been defined. This is not the case with the quasirandom structure and function guided synthesis methods of the present invention in which a connector polynucleotide (CPN) guides the synthesis of a molecule by calling for complementary connector polynucleotides (CCPNs) capable of hybridizing to the CPN. This is illustrated in Fig. 2.

Unlike template directed synthesis methods in which the sequence of codons of the template determines the sequence of anti-codons or transfer units hybridizing to the template, the final structure of a supramolecular complex comprising a plurality a CPNs and a plurality of CCPNs cannot readily be predicted in all cases prior to carrying out the quasirandom structure and function guided synthesis methods of the present invention.

The quasirandom structure and function guided synthesis methods of the present invention - being less deterministic than template directed synthesis methods relying exclusively on a predetermined codon sequence - has a number of advantages over template directed synthesis methods.

The individual molecules of the present invention are generated during or after the formation of a higher order polynucleotide complex comprising a plurality of connector polynucleotides (CPN's) and a plurality of complementary connector polynucleotides (CCPN's) of which at least some CPN's and/or CCPN's are carrying reactants such as e.g. functional entities/chemical moieties, wherein said reactants are either

precursor components to be used in the synthesis of the molecule (i.e. components which can be reacted, act as catalysers, be spatially rearranged, or otherwise altered in structure and/or function) and/or components which can otherwise be integrated into the synthesized molecule.

The association of two complementary connector polynucleotides through a connector polynucleotide ensures one or more of the following desirable characteristics:

A high reactivity between functional entities present on different CCPN's (because of a high proximity/local concentration of reactants such as functional entity reactive groups),

a controllable reactant reactivity (*i.e.* functional entity reactive groups of complementary connector polynucleotides of a complex react with each other, and not with functional entity reactive groups of complementary connector polynucleotides of other complexes), and

an efficient selection of desitable molecules is ensured through iterative cycles of screening and amplification of connector polynucleotides, optionally including one or more "shuffling" steps ("shuffling" in this context includes mixing of connector polynucleotides to obtain complexes e.g. comprising the same connector polynucleotides, but in new combinations, or located in different positions).

Further advantages of the present invention relate to desirable features of higher order hybridization complexes comprising a plurality of connector polynucleotides (CPN's) and complementary connector polynucleotides (CCPN's). The advantages include, among other things:

A desirable variability in the number of reactants which can be provided for the synthesis, *i.e.* the ability to vary the number of complementary connectors (CCPN's) for each molecule within a library, thus providing a high degree of flexibility in the generation of libraries of chemical compounds.

Libraries of e.g. 10^8 or more chemical compounds can be generated with a relatively low diversity of CCPN's - unlike libraries of a similar size generated from template

directed methods, which require a much higher number of anti-codons or transfer units to be used, as no variability can be achieved for the template directed methods.

A high variation in the degree of functionalization of scaffolds is possible, *i.e.* allowing diversification of branching degree.

It is possible to generate a library - and to further evolve the library - by exploiting CCPN "cross-talk", i.e. the ability of one CCPN reactant to preferably react with a subset of all available CCPN reactants.

The methods can employ a large set of scaffolds and allow a diverse set of attachments chemistries to be used for diversifying scaffolds or libraries of chemical compounds.

Inherent shuffling steps can be used for evolving scaffolds and chemical libraries, including steps in which connector polynucleotides are mixed to obtain complexes e.g. comprising the same connector polynucleotides, but in new combinations, or located in different positions.

Short oligonucleotides can be used in the methods of the present invention. This offers a cost effective means for generating large libraries. The oligonucleotides used in the methods of the present invention are much shorter than the often very long oligonucleotides used in prior art methods exploiting template directed synthesis of chemical compounds.

In a first aspect there is provided a method for synthesising a molecule comprising the steps of

i) providing a plurality of connector polynucleotides each capable of hybridizing to at least 1 complementary connector polynucleotide,

ii) providing a plurality of complementary connector polynucleotides selected from the group consisting of

a) complementary connector polynucleotides comprising at least 1 functional entity comprising at least 1 reactive group,

b) complementary connector polynucleotides comprising at least 1 reactive group,

c) complementary connector polynucleotides comprising at least 1 spacer region,

iii) hybridizing at least 2 complementary connector polynucleotides to at least 2 connector polynucleotides,

wherein at least 2 of said complementary connector polynucleotides comprise at least 1 functional entity comprising at least 1 reactive group,

wherein at least 1 of said complementary connector polynucleotides hybridizes to at least 2 connector polynucleotides, and

iv) reacting at least 2, such as 3 or more functional entity reactive groups by reacting at least 1 reactive group of each functional entity,

wherein the reaction of said functional entity reactive groups results in the formation of the molecule by covalently linking at least 2 functional entities provided by separate complementary connector polynucleotides.

In a further aspect there is provided a method for synthesising one or more molecule(s) comprising the steps of

i) providing a plurality of connector polynucleotides each capable of hybridizing to at least 1 complementary connector polynucleotide,

ii) providing a plurality of complementary connector polynucleotides selected from the group consisting of

a) complementary connector polynucleotides comprising at least 1 functional entity comprising at least 1 reactive group,

b) complementary connector polynucleotides comprising at least 1 reactive group,

c) complementary connector polynucleotides comprising at least 1 spacer region,

iii) hybridizing at least 2 complementary connector polynucleotides to at least 2 connector polynucleotides,

wherein at least 2 of said complementary connector polynucleotides comprise at least 1 functional entity comprising at least 1 reactive group,

wherein at least 1 of said complementary connector polynucleotides hybridizes to at least 2 connector polynucleotides, and

iv) reacting at least 2, such as 3 or more functional entity reactive groups by reacting at least 1 reactive group of each functional entity,

wherein the reaction of said functional entity reactive groups results in the formation of the molecule by covalently linking at least 2 functional entities provided by separate complementary connector polynucleotides,

wherein the molecule comprising covalently linked functional entities is linked to a the polynucleotide part of a complementary connector polynucleotide,

wherein the molecule does not comprise the linker and the polynucleotide part of said complementary connector polynucleotide,

wherein complementary connector polynucleotides hybridized to connector polynucleotides are not linked by covalent bonds,

wherein connector polynucleotides hybridized to complementary connector polynucleotides are not linked by covalent bonds, and

wherein the method does not involve ribosome mediated translation.

In a still further aspect there is provided a method for synthesising at least one molecule comprising the steps of

i) providing a plurality of connector polynucleotides each capable of hybridizing to at least 1 complementary connector polynucleotide,

ii) providing a plurality of complementary connector polynucleotides selected from the group consisting of

a) complementary connector polynucleotides comprising at least 1 reactant comprising at least 1 reactive group

b) complementary connector polynucleotides comprising at least 1 reactive group,

c) complementary connector polynucleotides comprising at least 1 spacer region,

iii) hybridizing at least 2 complementary connector polynucleotides to at least 2 connector polynucleotides,

wherein at least 2 of said complementary connector polynucleotides comprise at least 1 reactant comprising at least 1 reactive group,

wherein at least 1 of said complementary connector polynucleotides hybridizes to at least 2 connector polynucleotides, and

iv) synthesising the at least one molecule by reacting at least 2 reactants.

In a further aspect there is provided a method for synthesising at least one molecule comprising the steps of

i) providing a plurality of building block polynucleotides each capable of hybridizing to at least 1 other building block polynucleotide,

wherein said building block polynucleotides are selected from the group consisting of

a) building block polynucleotides comprising at least 1 reactant comprising at least 1 reactive group

b) building block polynucleotides comprising at least 1 reactive group,

c) building block polynucleotides comprising at least 1 spacer region,

ii) forming a hybridization complex comprising at least 4 building block polynucleotides,

wherein at least 2 of said building block polynucleotides comprise at least 1 reactant comprising at least 1 reactive group,

wherein at least 1 of said building block polynucleotide hybridizes to at least 2 other building block polynucleotides, and

iii) synthesising the at least one molecule by reacting at least 2 reactants.

In a still further aspect there is provided a method for synthesising a plurality of different molecules, said method comprising the steps of

i) providing a plurality of connector polynucleotides each capable of hybridizing to at least 1 complementory connector polynucleotide,

ii) providing a plurality of complementory connector polynucleotides selected from the group consisting of

a) complementory connector polynucleotides comprising at least 1 functional entity comprising at least 1 reactive group,

b) complementory connector polynucleotides comprising at least 1 reactive group,

c) complementory connector polynucleotides comprising at least 1 spacer region,

iii) hybridizing the plurality of connector polynucleotides and complementory connector polynucleotides, thereby forming a plurality of different hybridisation complexes, each hybridisation complex comprising at least 2 complementory connector polynucleotides and at least 2 connector polynucleotides,

wherein, for each of said hybridisation complexes,

at least 2 of said complementory connector polynucleotides comprise at least 1 functional entity comprising at least 1 reactive group, and

at least 1 of said complementory connector polynucleotides hybridizes to at least 2 connector polynucleotides, and

iv) reacting at least 2 functional entity reactive groups of each complex by reacting at least 1 reactive group of each functional entity,

wherein, for each hybridisation complex, the reaction of said functional entity reactive groups results in the formation of a different molecule by covalently linking at least 2 functional entities provided by separate complementory connector polynucleotides, thereby synthesising a plurality of different molecules.

In a still further aspect there is provided a method for identification of at least one molecule having desirable characteristics, said method comprising the steps of

i) targeting a plurality of different molecules to a potential binding partner, wherein the plurality of different molecules are a) synthesised by any of the methods cited herein for synthesising at least one molecule, or b) synthesised by the below mentioned method steps iii) and iv),

ii) selecting at least one of said molecules having an affinity for said binding partner,

iii) isolating connector polynucleotides from the selected molecules of step ii),

iv) optionally, hybridizing the connector polynucleotides isolated in step iii) to a plurality of complementory connector polynucleotides selected from the group consisting of

a) complementory connector polynucleotides comprising at least 1 functional entity comprising at least 1 reactive group,

b) complementory connector polynucleotides comprising at least 1 reactive group,

c) complementory connector polynucleotides comprising at least 1 spacer region,

reacting the functional entity reactive groups, thereby generating at least one molecule by linking at least 2 functional entities provided by separate complementory connector polynucleotides, and

performing steps i), ii), and iii) above for the at least one molecule generated in step iv), and

v) decoding the nucleic acid sequence of isolated connector polynucleotides to reveal the identity of functional entities that have participated in the formation of the molecule(s) having an affinity for said binding partner.

In a still further aspect there is provided a method for selecting at least one bifunctional molecule comprising a hybridisation complex linked to at least one molecule part comprising reacted reactants, such as covalently linked functional entities, wherein each complex comprises a plurality of connector polynucleotides (CPNs) and a plurality of complementary connector polynucleotides (CCPNs) having guided the synthesis of the molecule, wherein at least 2 of said CPNs and/or said CCPNs have each donated at least one reactant, such as at least one functional entity, to the method for synthesising the at least one molecule, wherein the complex comprises as least 1 CCPN hybridized to at least 2 CPNs, said method comprising the steps of targeting a plurality of the bifunctional molecules to a potential binding partner for the at least one molecule part of the bifunctional molecule linked by at least one linker to a CPN and/or a CCPN of the hybridization complex, wherein said binding partner has an affinity for the molecule part of the bifunctional molecule, and selecting at least one of said bifunctional molecules comprising at least one molecule part having an affinity for said binding partner. The method optionally comprises the further step of decoding the hybridisation complex, preferably by identifying the CPNs and/or the CCPNs forming the hybridisation complex, or part thereof, of the bifunctional molecule, and thereby identifying the molecule part of the bifunctional molecule. The decoding can involve ligating individual CPNs and/or ligating individual CCPNs of the hybridisation complex, optionally a ligation preceded by a polynucleotide extension reaction filling in any gaps between hybridised CPNs and/or hybridised CCPNs, amplifying the ligated CPNs and/or the ligated CCPNs, or amplifying at least part of the polynucleotide part of the ligated CPNs and/or the ligated CCPNs, sequencing the amplified part(s), and thereby determining the identity of the CPNs and/or CCPNs forming part of the hybridisation complex, or determining at least part of said identity allowing a conclusive identification of the individual CPNs and/or the individual CCPNs.

In yet another aspect there is provided a method for evolving a plurality of bifunctional molecules comprising a hybridisation complex linked to at least one molecule part comprising reacted reactants, such as covalently linked functional entities, wherein each complex comprises a plurality of connector polynucleotides (CPNs) and a plurality of complementary connector polynucleotides (CCPNs) having guided the synthesis of the molecule, wherein at least 2 of said CPNs and/or said CCPNs have each donated at least one functional entity to the method for synthesising the

of at least one molecule, wherein each complex comprises as least 1 CCPN hybridized to at least 2 CPNs, said method comprising the steps of selecting at least one bifunctional molecule, optionally by performing the immediately above-cited method for selecting at least one bifunctional molecule, isolating CPNs from said complex, optionally by ligating the CPNs and cleaving the ligation product with suitable restriction nucleases, thereby obtaining isolated CPNs, further optionally by performing a polynucleotide extension reaction prior to performing the ligation reaction in order to close any gaps between the CPNs, providing a plurality of CCPNs at least some of which comprise a reactant, such as a functional entity comprising a reactive group, hybridising said isolated CPNs and said plurality of provided CCPNs, reacting reactants, such as reacting functional entity reactive groups of said CCPNs comprising such groups, optionally repeating any one or more of the aforementioned steps, and evolving a plurality of different bifunctional molecules.

In a further aspect of the invention there is provided a bifunctional molecule obtainable by any of the methods of the invention and comprising a molecule part formed by reaction of reactants, such as functional entities, and a nucleic acid part formed by hybridisation between at least 2 complementory connector polynucleotides and at least 2 connector polynucleotides, including a nucleic acid part formed by hybridisation between at least the polynucleotide entity of 2 complementory connector polynucleotides and at least the polynucleotide entity of 2 connector polynucleotides.

In yet another aspect there is provided a composition of bifunctional molecules obtainable by any of the methods of the invention, wherein each member of the composition comprises a molecule part formed by reaction of reactants, such as functional entities, and a nucleic acid part comprising a hybridisation complex between at least the polynucleotide entity of 2 complementory connector polynucleotides and at least the polynucleotide entity of 2 connector polynucleotides.

There is also provided a hybridization complex comprising a plurality of connector polynucleotides and a plurality of complementory connector polynucleotides, wherein the complex comprises as least 2 complementory connector polynucleotides hybridized to at least 2 connector polynucleotides. The hybridisation complex can be regarded as an intermediate product in the process of generating

the above-mentioned bifunctional molecule(s). Accordingly, a hybridisation complex can be present prior to or during molecule synthesis, but once the molecule has been synthesised, it forms part of a bifunctional molecule further comprising the CPNs and CCPNs forming part of the hybridisation complex of the bifunctional molecule.

In yet another aspect there is provided a supramolecular complex comprising at least one molecule comprising covalently linked functional entities and a plurality of connector polynucleotides (CPNs) and a plurality of complementary connector polynucleotides (CCPNs), wherein at least some of said CPNs and/or CCPNs have donated functional entities to the synthesis of the at least one molecule, wherein the complex comprises as least 1 CCPN hybridized to at least 2 CPNs. In a further aspect there is provided a plurality of such supramolecular complexes.

Definitions

At least 1 single complementary connector polynucleotide (CCPN) hybridizes to at least 2 connector polynucleotides (CPN): The hybridization events leading to the formation of the supramolecular complex can occur simultaneously or sequentially in any order as illustrated in Fig. 2.

A bifunctional molecule comprises a (final) molecule part and a hybridisation complex part. The hybridisation complex part of the bifunctional molecule comprises at least 2 CCPNs the polynucleotide part of which (individual CCPN) is hybridised to the polynucleotide part of at least 1 CPN, wherein at least some of said hybridised CPNs and/or CCPNs have provided their reactants, such as functional groups, to the method for synthesising the at least one molecule linked to the hybridisation complex of the bifunctional molecule.

Branched CPN: Connector polynucleotide comprising one or more branching points connecting linear or branched polynucleotides.

Building block polynucleotide: Generic term for a polynucletide part linked to either a) a reactant such as a functional entitity comprising at least one reactive group (type I BBPN), or b) a reactive group (in the absence of a reactant or functional en-

tity) (type II BBPN), or the BBPN can simply comprise a polynucleotide part comprising a spacer region for spacing e.g. functional entities of other BBPNs (type III BBPN). The term building block polynucleotide thus includes CPNs and CCPNs irrespective of their type.

Complementary connector polynucleotide (CCPN): Part of a supramolecular complex comprising a plurality of CPNs and a plurality of CCPNs as illustrated in Fig. 2. A CCPN comprises a polynucleotide part which can be linked to either a) a reactant such as a functional entitity comprising at least one reactive group (type I CCPN), or b) a reactive group (in the absence of a reactant or functional entity) (type II CCPN), or the CCPN can simply comprise a polynucleotide part comprising a spacer region for spacing e.g. functional entities of other CCPNs (type III CCPN). When the polynucleotide part of a CCPN is linked to a reactant, such as a functional entity comprising at least one reactive group, or in the functional entity being covalently linked to another functional entity, or part thereof, the CCPN acts as a "donor CCPN" or as an "acceptor CCPN" and thus takes part in the method for synthesising the at least one molecule. In some embodiments, some CCPNs will be "donor CCPNs" donating functional entities to the synthesis of a molecule comprising covalently linked functional entities, whereas at least one other CCPN will be an "acceptor CCPN", or a CPN will be an "acceptor CPN". A method for synthesising at least one molecule exploiting one or more "donor CCPNs" comprising at least one reactant, such as at least one functional group, does not exclude using - in the same method - at least one "donor CPN" comprising at least one reactant, such as at least one functional group. The covalent or non-covalent bond between a functional entity and a polynucleotide part of a "donor CCPN" can be cleaved before, during, or after the synthesis and formation of the molecule comprising reacted reactants, such as covalently linked functional entities. A covalent bond will be generated between reactants or functional entities associated with an acceptor CCPN, or an acceptor CPN, during the synthesis of the molecule comprising reacted reactants, such as covalently linked functional entities. The synthesis and formation of molecules each comprising covalently linked functional entities is thus in one embodiment a result of both i) formation of covalent bonds linking functional entities present on acceptor CCPNs, and ii) cleavage of covalent bonds linking functional entities and polynucleotides of donor CCPNs. Once a molecule has been synthesised in this fashion,

no donor CCPNs will be linked to each other by covalent bonds, and no covalent bonds will link individual donor CCPNs and an acceptor CCPN.

Connector polynucleotide: Part of a supramolecular complex comprising a plurality of CPNs and a plurality of CCPNs as illustrated in Fig. 2. A CPN guides the synthesis of a molecule comprising covalently linked functional entities by "calling" for CCPNs capable of hybridizing to the CPN. In some embodiments, it is preferred that the CPNs comprise only a polynucleotide part, and no reactant (or functional entity) or reactive group(s) (CPN type III). However, in other embodiments, the polynucleotide part of a CPN can be linked to at least one reactant (or functional entity) comprising at least one reactive group (CPN type I), or the polynucleotide part of a CPN can be linked to a reactive group (in the absence of a reactant or functional entity) (CPN type II).

Decoding: The nucleic acid part of a CPN or a CCPN harbours information as to the identity of the corresponding reactant or functional entity linked to the nucleic acid part of the CPN or the CCPN. Following a selection step the functional entities which have participated in the formation of the encoded molecule can be identified. The identity of a molecule can be determined if information on the chemical entities, the synthesis conditions and the order of incorporation can be established.

The nucleic acid part of the CCPNs or CPNs of successful hybridisation complexes can be decoded separately, or the various nucleic acid strands can be ligated together prior to decoding. In one embodiment of the invention individual CPNs are ligated together prior to decoding to ease the handling of the various informative nucleic acid strands, i.e. the polynucleotide part of the individual CPNs having participated in the synthesis of the at least one molecule. A ligation product between individual CPNs, or between individual CCPNs, of a selected bifunctional molecule is referred to below as an identifier sequence. It may be sufficient to obtain information on the chemical structure of the various functional entities that have participated in the synthesis of the at least one molecule in order to deduce the full structure of the molecule, as structural constraints during the formation can aide the identification process. As an example, the use of different kinds of attachment chemistries may ensure that a chemical entity on a building block can only be transferred to a certain position on a scaffold. Another kind of chemical constrains may be present due to

steric hindrance on the scaffold molecule or the functional entity to be transferred. In general however, it is preferred that information can be inferred from the identifier sequence that enable the identification of each of the functional entities that have participated in the formation of the encoded molecule along with the point in time in the synthesis history when the chemical entities have been incorporated in the (nascent or intermediate) molecule.

Although conventional DNA sequencing methods are readily available and useful for this determination, the amount and quality of isolated bifunctional molecule hybridisation complexes linked to a molecule having the desired property may require additional manipulations prior to a sequencing reaction. Where the amount is low, it is preferred to increase the amount of the identifier sequence by polymerase chain reaction (PCR) using PCR primers directed to primer binding sites present in the identifier sequence. In addition, the quality of the library may be such that multiple species of different bifunctional molecules are co-isolated by virtue of similar capacities for binding to a target. In cases where more than one species of bifunctional molecule are isolated, the different isolated species can suitably be separated prior to sequencing of the identifier oligonucleotide.

Thus in one embodiment, the different identifier sequences of the isolated bifunctional complexes are cloned into separate sequencing vectors prior to determining their sequence by DNA sequencing methods. This is typically accomplished by amplifying all of the different identifier sequences by PCR, and then using unique restriction endonuclease site(s) on the amplified product to directionally clone the amplified fragments into sequencing vectors. The cloning and sequencing of the amplified fragments is a routine procedure that can be carried out by any of a number of molecular biological methods known in the art.

Alternatively, the bifunctional complex or the PCR amplified identifier sequence can be analysed in a microarray. The array may be designed to analyse the presence of a single codon or multiple codons in a identifier sequence.

Functional entity: Part of a CPN or a CCPN. Functional entities comprise at least one reactive group. The functional entity comprises a part or an intermediate of the molecule to be synthesised. A functional entity can also comprise the product of a

reaction having previously taken place between separate functional entities, i.e. the term also applies to intermediate products being generated prior to or during the synthesis of the molecule.

The functional entity of a CPN or CCPN serves the function of being a precursor for the structural entity eventually appearing on the encoded molecule. Therefore, when it is stated in the present application that a functional entity is linked to another functional entity through the reaction of the reactive groups of respective functional entities, it is to be understood that not necessarily all the atoms of the original functional entity is to be found on the final molecule having been synthesised. Also, as a consequence of the reactions involved in the linking, the structure of the functional entity can be changed when it appears on the encoded molecule. Especially, the cleavage resulting in the release of the functional entity may generate reactive group(s) which in a subsequent reaction can participate in the formation of a connection between the (nascent or intermediate) molecule and a further functional entity. Furthermore, two or more functional entities may generate an intermediate which can be reacted with a third (or further) functional entity to form a nascent or final molecule.

The connection or linking between functional entities or, alternatively, a functional entity and a nascent encoded molecule, is aided by one or more reactive groups of the functional entities. The reactive groups may be protected by any suitable protecting groups which need to be removed prior to the linking of the functional entities. Dependent on the reaction conditions used, the reactive groups may also need to be activated. A functional entity featuring a single reactive group may suitably be used i.a. in the end positions of polymers or to be reacted with a scaffold, whereas functional entities having two or more reactive groups intended for the formation of linkage between functional entities, are typically present as scaffolds or in the body part of a polymer. A scaffold is a core structure, which forms the basis for creating multiple variants of molecules based on the same set of functional entities to be reacted in different combinations in order to generate the variants . The variant forms of the scaffold is typically formed through reaction of reactive groups of the scaffold with reactive groups of other functional entities, optionally mediated by fill-in groups or catalysts, under the creation of a covalent linkage.

Functional entity reactive group: Each functional entity comprises at least one reactive group the reaction of which with a reactive group of a separate functional entity results in the formation of covalently linked functional entities, or part thereof.

A reactive group of a functional entity may be capable of forming a direct linkage to a reactive group of another functional entity, or a nascent or intermediate molecule, or a reactive group of a functional entity may be capable of forming a connection to a reactive group of another functional entity through a bridging fill-in group. It is to be understood that not all the atoms of a reactive group are necessarily maintained in the connection formed. Rather the reactive groups are to be regarded as precursors for the linkage formed.

Hybridization complex: Plurality of CPN's hybridised to a plurality of CCPN's, wherein one or more reactants or functional entities or intermediate molecules can be linked to one or more CPN's and/or CCPN's. Accordingly, a single intermediate molecule can be linked to either a CPN and/or a CCPN, and different reactants or functional entities or intermediate molecules can be linked to the same or different CPN(s) or CCPN(s). Once the final molecule has been formed, the term hybridisation complex is no longer used, instead, the term bifunctional molecule comprising a (final) molecule part and a hybridion complex part is used. The overlap of complementary polynucleotides of CPNs and CCPNs hybridising to one another is preferably 4 or more nucleotides, such as e.g. 6 nucleotide overlaps, for example overlaps of 10-12 nucleotides.

Linear CPN: CPN comprising a sequence of covalently linked nucleotides.

Molecule: Molecule comprising covalently linked functional entities, or the molecule being the reaction product when reactive groups of different (i.e. separate) functional entities are reacted and functional entities are joined together or linked to a scaffold. The molecule can be linked to the polynucleotide part of a CCPN by a linker. In one embodiment, neither the linker nor the polynucleotide part of the CCPN forms part of the molecule. The formation of a molecule involves in one embodiment the transfer of at least one functional entity, or part thereof, a) from one or more CCPN(s) to one or more separate CCPN(s), and/or b) from one or more CPN(s) to one or more separate CPN(s), and/or c) from one or more CPN(s) to one or more CCPN(s),

and/or d) from one or more CCPN(s) to one or more CPN(s), preferably by reacting at least 2, such as at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 functional entity reactive groups in order to synthesise the molecule. Either before, during, or after the transfer of the at least one functional entity from one building block polynucleotide to another, a covalent bond between the at least one functional entity and the polynucleotide of the donor CCPNs is cleaved. Once a molecule has been synthesised in this fashion, no donor CCPNs will be linked to each other by covalent bonds, and no covalent bonds will link individual donor CCPNs and an acceptor CCPN.

Other reactive groups: Groups the reaction of which does not result in the formation of a molecule comprising covalently linked functional entities. The reaction of other reactive groups does not involve the donation of a functional entity or a part thereof from one CCPN to another CCPN.

Plurality: At least 2, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, such as 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, for example, 200, 300, 400, 500, 600, 700, 800, 900, 1000, such as more than 1000.

Reactant: Precursor moiety for a structural unit in the synthesised molecule. The reaction of reactants result in the formation of at least one molecule in accordance with the methods of the present invention.

Reacting functional entity reactive groups: A molecule is generated by reactions involving functional entity reactive groups. Reacting functional entity reactive groups of separate functional entities results in linking the functional entities or a part thereof by covalent bonds. Types of reactive groups and types of reactions involving such reactive groups are listed in Fig. 23. The listing is merely exemplifying and not exhaustive.

Reactive group: Activatable part of e.g. a reactant, such as a functional entity, i.e. a (reactive) group forming part of, being integrated into, being linked to, or otherwise associated with, a building block polynucleotide of type I as designated herein. A reactive group, such as e.g. a catalyst, can also occur on its own without forming part

of, being integrated into, being linked to, or otherwise associated with, a reactant, such as a functional entity. In the latter case the reactive group is linked to the polynucleotide part of a building block polynucleotide of type II as designated herein.

Spacer region: Region on a CPN or CCPN capable of separating and/or spatially organising functional entities located on adjacently positioned CPNs or CCPNs in a hybridisation complex. In one embodiment the spacer region is the region of a building block polyncleotide not hybridised to another building block polynucleotide. The polynucleotide part of both CPNs and CCPNs can comprise a spacer region, optionally in the absence of a functional entity or a reactive group linked to said polynucleotide part. In some embodiments, a building block polynucleotide comprising a spacer region in the polynucleotide part of the building block polynucleotide does not comprise a reactant or a functional entity or a reactive group (participating in molecule formation) linked to said polynucleotide part of said building block polynucleotide. However, building block polynucleotides comprising such reactants or functional entities or reactive groups linked to the polynucleotide part of the building block polynucleotide may further comprise a spacer region, such as e.g. a region of the polynucleotide part of the building block polynucleotide which does not hybridise to the polynucleotide part of other building block polynucleotides. In such embodiments, it will be understood that CPNs of type III and CCPNs og type III (as designated herein elsewhere) do not also comprise one or more reactants, or one or more functional entities, or one or more reactive groups participating in molecule formation. Spacer regions can be designed so that they are capable of self-hybridization and hair-pin structure formation. Preferred "spacer regions" are polynucleotides to which no functional entities and no reactive groups are attached.

Zipper box: Linkers linking functional entities to e.g. the polynucleotide part of a CPN or a CCPN can comprise a "zipper box". Two linkers may be provided with a zipper box, i.e. a first linker comprises a first part of a molecule pair being capable of reversible interaction with a second linker comprising the second part of the molecule pair. Typically, the molecule pair comprises nucleic acids, such as two complementary sequences of nucleic acids or nucleic acid analogs. In a certain aspect, the zipper domain polarity of the CCPN harbouring the first linker attached to the first functional entity is reverse compared to the zipper domain polarity of the CCPN harbouring the second functional entity. Usually, the zipping domain is proximal to the func-

tional entity to allow for a close proximity of the functional entities. In preferred embodiments, the zipping domain is spaced form the functional entity with no more than 2 nucleic acid monomers. Typically, the zipping domain sequence comprises 3 to 20 nucleic acid monomers, such as 4 to 16, and preferably 5 to 10, depending on the conditions used.

The annealing temperature between the nucleic acid part of the CCPN and a CPN is usually higher than the annealing temperature of the zipper box molecule pair to maintain the hybridisation complex during the reaction. Usually, the difference between the annealing temperatures is 10°C, such as 25 °C, or above. In a certain embodiment of the invention, the conditions during assembling of the hybridisation complex includes a concentration of the CCPN and CPN which is higher than the concentration during reaction to allow for optimal dimerisation conditions for the two parts of the molecule pair. The concentration during the assembly of the hybridisation complex is in a preferred aspect at least 10 times higher compared to the concentration used for dimerisation of the to parts of the molecule pair. In a certain aspect, the reaction step is performed by altering the temperature below and above the annealing temperature of the zipping domain, however ensuring that the hybridisation complex retains its integrity.

Brief Description of the Figures

Figure 1.

The figure illustrates different examples of complementary connector polynucleotides (CCPN's).

A.) A CCPN containing an oligonucleotide/polynucleotide sequence, a linker and a functional entity carrying one or more reactive groups. The linker may optionally be cleavable and may comprise an oligonucleotide, a natural or unnatural peptide or a polyethyleneglycol (PEG), a combination thereof or other linkers generally used in organic synthesis, combinatorial chemistry or solid phase synthesis.

B.) Similar to A with a different positioning of the reactive group.

C.) A combination of type A and type B.

D.) This CCPN only contains a reactive group and not a functional entity in the sense of types A, B and C.

E.) A spacer CCPN without functional entity.

Figure 2.

The figure illustrates the overall concept of the present invention. A set of CCPN's are mixed either sequentially or simultaneously with a set of CPN's, whereby at least two complementary connector polynucleotides hybridize to at least two connector polynucleotides, wherein at least two of said complementary connector polynucleotides comprise at least one functional entity comprising at least one reactive group, and wherein at least one of said complementary connector polynucleotides hybridizes to at least two connector polynucleotides.

In the next step, reaction occurs between reactive groups on functional entities, whereby a molecule is obtained by linking at least two functional entities, each provided by a separate complementary connector polynucleotide, by reacting at least one reactive group of each functional entity. If a number of such hybridization complexes are formed a number of molecules will be synthesized. If this is performed in one tube, a mixed library of compounds is prepared. Such molecules, attached to a CCPN or a number of CCPN's, form together with the CPN's, to which they hybridize, a complex.

The library of compounds/complexes may then be assayed for specific properties such as e.g. affinity or catalytic activity, and compounds/complexes with such activity may be isolated. The CPN's and/or CCPN's of such complexes may be isolated and amplified. Such amplified CPN's may go into further rounds of library generation, whereby a new library of compounds/complexes will be formed, a library which is enriched in molecules with properties corresponding to the properties assayed for.

Figure 3.

The figure illustrates a set of different molecules which may be formed by the process of the present invention through the steps described above for Figure 2. The figure serves only for illustrative purposes and is not in any way intended to limit the scope of the present invention.

Figure 4.

The figure illustrates various hybridisation complexes comprising CPNs and CCPNs. Reactants or functional entities the reaction of which generates the at least one molecule is illustrated by capital letters (X, Y, Z, etc.). For illustration purposes the functional entities remain associated with the "donor CCPNs" (or "donor CPNs"), however, the reactants can react prior to, during or after the formation of the hybridisation complexes indicated in the figure. Once the reactants have reacted and the molecule has been generated, a bifunctional molecule is formed. The reaction of reactive groups can involve e.g. reacting at least one reactive group of each reactant or functional entity, or it can involve reacting one reactive group of a plurality of reactants with a plurality of reactive groups of a single reactant, typically a scaffold moiety. The hybridization complexes can be linear or circular as illustrated in the figure. The CPNs and/or the CCPNs can be linear or branched. The circular symbol with an x indicates a CPN/CCPN in an orientation perpendicular to the plane of the paper.

Figure 5.

The figure illustrates a further set of examples of CCPN's, wherein the linker maybe placed at one end of the polynucleotide sequence. In examples E. and F. the CCPN's neither carries a functional entity nor a reactive group. In example E. the CCPN may be capable of self association e.g. through complementary nucleotide sequences, whereby hybridization can occur. In example F., part of the CCPN loops out upon association such as e.g. hybridization with a CPN. In this example no self association occurs.

Figure 6.

The figure illustrates one embodiment of the concept described and shown in Figure 2. In this embodiment some or all polynucleotides of CCPN's are ligated together and some or all polynucleotides of CPN's are ligated together. Depending on the

number of CCPN's and CPN's in each individual complex formed, different lengths of ligated CPN's may be isolated. Alternatively, the ligated products are not isolated, but rather is followed by an amplification step by e.g. PCR, which will selectively amplify the ligated CPNs. These ligated CPN's may undergo PCR and be analysed by e.g. sequencing. The ligated CPN's may be fragmentalised again, e.g. through the use of restriction enzymes.

Figure 7.

As in Figure 6 wherein some or all the CPN's in each complex are ligated together whereas the CCPN's are not. This may be achieved e.g. if a gap between the polynucleotide sequences of CCPN's exists.

Figure 8.

As in Figure 6 wherein some or all the CCPN's in each complex are ligated together whereas the CPN's are not. This may be achieved e.g. if a gap between the polynucleotide sequences of CPN's exists. In this embodiment fragmentalisation of ligated CPN's is not performed during the process.

Figure 10.

As in Figure 6 for the first round of library formation, however, with the optional omission of fragmentalisation of ligated CPN's in second and later rounds of library formation and with the optional ligation of CCPN's in the second and later rounds of library formation. If fragmentalization (not shown) is performed during rounds libraries will be formed in such rounds as shown for the 1st round of library formation.

Figure 11.

As in Figure 6, except that the steps of ligation and reaction of functional entities have been interchanged, such that reaction of functional entities occurs prior to ligation. Here, the ligation serves as an introductory step for the amplification of the CPNs and CCPNs (by e.g. PCR). Alternatively, a "ligated-CPN product" and its amplification may also be obtained by performing a PCR after the reaction step, without the addition of primers. This will lead to the assembly of the various CPNs into one strand; the product can then be amplified by the addition of external primers.

Figure 12.

As in Figure 6, wherein the steps of ligation and selection have been changed, such that selection occurs prior to ligation. As in Figure 11, instead of ligating, PCR without external primers can be performed, followed by PCR including external primers.

Figure 13.

As in Figure 6, wherein some CPN's are capable of self hybridization, whereby CCPN's and CPN's in each complex may be linked. The ligation product following selection may optionally be treated with e.g. restriction enzymes to allow the ligated CPN's to be isolated or non-ligated CPN's to be isolated through partial or total fragmentalisation.

Figure 14.

As in Figure 8, wherein at least one CPN in each complex is capable of self hybridization, whereby CCPN's and some or all CPN's in each complex may be linked. In this example only one terminal CPN is capable of self hybridization and is ligated to the CCPN's. This setting may allow an easy separation of CPN's from CCPN's.

Figure 15.

The figure illustrates a set of different molecules which may be formed by the process of the present invention through the steps described above. In this example where CPN's have been ligated together and CCPN's have been ligated together. The figure serves only for illustrative purposes and is not in any way intended to limit the scope of the present invention.

Figure 16-19.

The figures illustrates further examples of CPN and CCPN complexes with or without ligational steps and with (a) and without (b) terminal oligonucleotide overhangs.

Figure 20.

The figure illustrates different CPN/CCPN complexes, wherein the some or all CPN's carry a reactive group or a functional entity comprising one or more reactive groups.

Figure 21.

The figure illustrates the principle of a zipperbox. The zipperbox is a region optionally comprising an oligonucleotide sequence where said region is capable of hybridizing to another zipperbox, wherein this second zipperbox optionally comprises an oligonucleotide sequence complementary to the first zipperbox. The zipperbox may be situated on a CPN or a CCPN. Upon hybridization of two zipperboxes, the proximity between functional entity reactive groups increases, whereby the reaction is enhanced.

By operating at a temperature that allows transient interaction of complementary zipperboxes, functional entity reactive groups are brought into close proximity during multiple annealing events, which has the effect of reactive groups in close proximity in a larger fraction of the time than otherwise achievable. Alternatively, one may cycle the temperature between a low temperature (where the zipper boxes pairwise interacts stably), and a higher temperature (where the zipper boxes are apart, but where the CCPN/CPN complex remains stable. By cycling between the high and low temperature several times, a given reactive group is exposed to several reactive groups, and eventually will react to form a bond between two function entities through their reactive groups.

Figure 22.

The figure illustrates how different CPN and CCPN complexes may form by a self-fassembly process through cross talk between CPN's and CCPN's. The figure only illustrates two paths, but the illustration is not intended to limit the invention hereto. The complexes may form through the mixing of all components in one step or through the stepwise addition of CPN's and CCPN's in each step,

Figure 23.

The figure illustrates reaction types allowing simultaneous reaction and linker cleavage.Different classes of reactions are shown which mediate translocation of a functional group from one CCPN (or CPN (not illustrated)) to another, or to an anchorage CCPN. The reactions illustrated are compatible with simultaneous reaction and linker cleavage, *i.e.* one functional entity is transferred (translocated) directly from one CCPN (or CPN (not illustrated)) onto another CCPN (or CPN (not illustrated)) without the need of subsequent and separate linker cleavage through the application of further new conditions allowing for such.

(A) Reaction of nucleophiles with carbonyls. As a result of the nucleophilic substitution, the functional group (entity) R is translocated to the CCPN initially carrying the nucleophile.

(B) Nucleophilic attack by the amine on the thioester leads to formation of an amide bond, in effect translocating the functional group R of the thioester to the other CCPN.

(C) Reaction between hydrazine and β-ketoester leads to formation of pyrazolone, in effect translocating the R and R' functional groups to the other CCPN.

(D) Reaction of hydroxylamine with β-ketoester leads to formation of the isoxazolone, thereby translocating the R and R' groups to the other CCPN.

(E) Reaction of thiourea with β-ketoester leads to formation of the pyrimidine, thereby translocating the R and R' groups to the other CCPN.

(F) Reaction of urea with malonate leads to formation of pyrimidine, thereby translocating the R group to the other CCPN.

(G) Depending on whether Z = O or Z = NH, a Heck reaction followed by a nucleophilic substitution leads to formation of coumarin or quinolinon, thereby translocating the R and R' groups to the other CCPN.

(H) Reaction of hydrazine and phthalimides leads to formation of phthalhydrazide, thereby translocating the R and R' groups to the other CCPN.

(I) Reaction of amino acid esters leads to formation of diketopiperazine, thereby translocating the R group to the other CCPN.

(J) Reaction of urea with α-substituted esters leads to formation of hydantoin, and translocation of the R and R' groups to the other CCPN.

(K) Alkylation may be achieved by reaction of various nucleophiles with sulfonates. This translocates the functional groups R and R' to the other CCPN.

(L) Reaction of a di-activated alkene containing an electron withdrawing and a leaving group, whereby the alkene is translocated to the nucleophile carrying CCPN.

(M) Reaction of disulfide with mercaptane leads to formation of a disulfide, thereby translocating the R' group to the other CCPN.

(N) Reaction of amino acid esters and amino ketones leads to formation of benzodiazepinone, thereby translocating the R group to the other CCPN.

(O) Reaction of phosphonium salts with aldehydes or ketones leads to formation of substituted alkenes, thereby translocating the R" group to the other CCPN.

(P) Reaction of phosphonates with aldehydes or ketones leads to formation of substituted alkenes, thereby translocating the R" group to the other CCPN.

(Q) The principle of translocation of e.g. aryl groups from one CCPN to another CCPN.

(R) Reaction of boronates with aryls or heteroaryls results in transfer of an aryl group to the other CCPN (to form a biaryl).

(S) Reaction arylsulfonates with aryl groups bound as Boron derivatives leads to transfer of the aryl group.

(T) Biaryl formation through translocation of one aryl group to another CCPN.

(U) Arylamine formation (e.g. Hartwig/Buchwald type of chemistry) through *N*-arylation, *i.e.* transfer of aryl groups to CCPN's carrying amino groups.

(V) As U using hypervalent iodonium derivatives.

(W)

(X) Reaction of boronates with vinyls (or alkynes) results in transfer of an aryl group to the other CCPN to form a vinylarene (or alkynylarene).

(Y) Reaction between aliphatic boronates and arylhalides, whereby the alkyl group is translocated to yield an alkylarene.

(Z) Transition metal catalysed alpha-alkylation through reaction between an enolether and an arylhallide, thereby translocating the aliphatic part.

(AA) Condensations between e.g. enamines or enolethers with aldehydes leading to formation of alpha-hydroxy carbonyls or alpha,beta-unsaturated carbonyls. The reaction translocates the nucleophilic part.

(AB) Alkylation of alkylhalides by e.g. enamines or enolethers. The reaction translocates the nucleophilic part.

(AC) [2+4] cycloadditions, translocating the diene-part.

(AD) [2+4] cycloadditions, translocating the ene-part.

(AE) [3+2] cycloadditions between azides and alkenes, leading to triazoles by translocation of the ene-part.

(AF) [3+2] cycloadditions between nitriloxides and alkenes, leading to isoxazoles by translocation of the ene-part.

Figure 24.

The figure illustrates pairs of reactive groups (X) and (Y), and the resulting bond (XY).

A collection of reactive groups and functional entity reactive groups that may be used for the synthesis of molecules are shown, along with the bonds formed upon their reaction. After reaction, linker cleavage may be applied to release one of the functional entities, whereby the transfer of one functional entity from one CCPN to another is effectuated.

Figure 25.

The composition of linker may be include derivatives of the following, but is not limited hereto:

- Carbohydrides and substituted carbohydrides
- Vinyl, polyvinyl and substituted polyvinyl
- Acetylene, polyacetylene
- Aryl/Hetaryl, polyaryl/hetaryl and substituted polyaryl/polyhetaryl
- Ethers, polyethers such as e.g. polyethyleneglycol and substituted polyethers
- Amines, polyamines and substituted polyamines
- Double stranded, single stranded or partially double or single stranded natural and unnatural polynucleotides and substituted double stranded, single stranded or partially double stranded natural and unnatural polynucleotides such as but limited to DNA, RNA, LNA, PNA, TNA
- Polyamides and natural and unnatural polypeptides and substituted polyamides and natural and unnatural polypeptides
- Phosphate containing linkers
- Any combination of the above

Linkers may be cleavable or non-cleavable. The figure illustrates cleavable linkers, conditions for their cleavage, and the resulting products are shown.

Figure 26.

The figure illustrates different examples of the formation of CCPN's carrying functional entities. Reactions and reagents are shown that may be used for the coupling of functional entities to modified oligonucleotides (modified with thiol, carboxylic

acid, halide, or amine), without significant reaction with the unmodified part of the oligonucleotide or alternatively, connective reactions for linkage of linkers to complementing elements. Commercially, mononucleotides are available for the production of starting oligonucleotides with the modifications mentioned.

Figure 27.
The figure illustrates the hair-pin oligo set-up.

Figure 28.
The figure illustrates the polyacrylamide gel analysis described in more detail in example 2A. The arrow indicates the cross-link product of the AH251 oligo and the radioactively labelled AH202 oligo. The cross-linked product has slower mobility in the gel than the labelled, non-reacted AH202 oligo.

Figure 29.
The figure illustrates the polyacrylamide gel analysis described in more detail in example 2B. The arrow indicates the cross-link product of the AH251 oligo and the radioactively labelled AH202 oligo. The cross-linked product has slower mobility in the gel than the labelled, non-reacted AH202 oligo.

Figure 30.
Encoding scheme for the synthesis of a small molecule from four encoded units (corresponding to CCPN0, CCPN1, CCPN2, and CCPN3), using a circular oligonucleotide CCPN/CPN-complex. This scheme is employed in example 2H; the first part of the scheme is employed in example 2G.

Figure 31. This figure shows the proposed circular structure, as well as gives an overview of the different oligos CPN T1, CCPN T2 and CPN T3 used in examples 2C-2H, and the CCPN0, CCPN1, CCPN2 and CCPN3 oligos carrying the functional entities. The insert shows the oligo set-ups used in the positive control reaction.

Figure 32. The polyacrylamide gel analysis of example 2C.
The arrow indicates the cross-link product of the AH381 or AH270 oligo with the radioactively labelled AH202 oligo. The cross-linked product has slower mobility in the gel than the labelled, non-reacted AH202 oligo.

Figure 33. The polyacrylamide gel analysis of example 2D.
The arrow indicates the cross-link product of the AH381 oligo with the radioactively labelled AH155 or AH272 oligos. The cross-linked product has slower mobility in the gel than the labelled, non-reacted AH155 or AH272 oligos.

Figure 34. The polyacrylamide gel analysis of example 2E.
The arrow indicates the cross-link product of the AH381 oligo with the radioactively labelled AH155 or AH272 oligos. The cross-linked product has slower mobility in the gel than the labelled, non-reacted AH155 or AH272 oligos.

Figure 35. The polyacrylamide gel analysis of example 2F.
The arrow indicates the cross-link product of the AH381 oligo with the radioactively labelled AH155 oligo. The cross-linked product has slower mobility in the gel than the labelled, non-reacted AH155 oligo.

Figure 36. The figure shows the proposed complex of example 2H.
The dotted circle highlights a part of the structure, consisting of 3 CCPNs and 2 CPNs, where one CCPN carries a functional entity and anneals to two CPNs.

Figure 37. Nitro phenol esters used in example 2G and 2H.
Structures and yields are given.

Detailed Description of the Invention

The method of the present invention have several advantages over template directed synthesis methods. As described below the methods of the present invention can be distinguished from well known methods such as e.g. ribosome mediated translation and ligation of polynucleotides.

In one embodiment of the present invention, the methods for synthesizing at least one molecule does not employ - for the purpose of synthesising the at least one molecule - the formation of a double stranded polynucleotide comprising complementary nucleotide stands obtained by joining or ligating end-positioned nucleotides by enzymatic reaction(s) or by chemical ligation using other reactive groups than 5'-

phophate groups and 3'-hydroxy groups employed by e.g. ligase catalysed reactions disclosed in standard text books (for chemical ligation, see e.g. by Bruick et al. (1997) and Gryaznov and Letsinger (1993)).

Rather, the method is directed to reacting functional entity reactive groups and thereby generating at least one small molecule, or a polymer molecule, by transferring functional entities or parts thereof from one or more donor CCPNs and/or donor CPNs to at least one acceptor CCPN or at least one acceptor CPN. A plurality of functional entities are preferably transferred from a plurality of donor CPPNs to a single (ultimate) acceptor CPPN. Functional entity reactive groups can react chemically or be enzymatically catalysed.

The end-product of the synthesis methods of the present invention is in one embodiment a molecule consisting of functional entities initially carried by CPN's and/or CCPN's. The molecule can also be obtained by reacting reactants provided by donor CPPNs and/or donor CPNs. The molecule is in one embodiment linked to the polynucleotide part of a CPN or a CCPN.

When the methods of the invention relate to functional entities carried primarily by CCPNs, a single functional entity can e.g. be transferred from each of a plurality of donor CPPNs to at least one acceptor CPPN, or more than one functional entity can be transferred from some or all of said donor CPPNs to an acceptor CPPN. When reactants or functional entities are donated to a scaffold, a plurality of reactive groups of said scaffold (e.g. a plurality of reactive groups of a single reactant or a single functional entity) will react with one or more reactive groups of the plurality of reactants or functional entities taking place in the formation of the scaffolded molecule.

The plurality of scaffold reactive groups involved in the formation of a scaffolded molecule can be e.g. at least 2, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 or more reactive groups. In one embodiment, the number of reactants or functional entities capable of reacting with the scaffold reactive groups is limited to the total number of scaffold reactive groups available for reaction with said reactants or functional entities linked to the polynucleotide part of donor CCPNs or donor

CPNs. Independently of the number of scaffold reactive groups, at least 2, such as e.g. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 reactants can react with the scaffold reactive groups when a scaffolded molecule is being formed in accordance with the methods of the present invention, wherein each reactant is preferably donated (provided) by a separate donor building block polynucleotide.

Independently of the number of scaffold reactive groups and independently of the number of reactants reacted, the number of donor building block polynucleotides in the hybridisation complex having provided reactants - directly or indirectly (i.e. several reactions having already taken place before a once or twice or further reacted reactant reacts with a scaffold reactive group) - for the synthesis of the scaffolded molecule can be anything in the range of from 2 to 25, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, for example from 2 to 8, such as from 2 to 6, for example from 2 to 5, such as from 2 to 4, for example from 3 to 25, such as from 3 to 20, for example from 3 to 15, such as from 3 to 10, for example from 3 to 8, such as from 3 to 6, for example from 3 to 5, such as 3 or 4. The total number of different donor building block polynucleotides present for the synthesis of different scaffolded molecules can of course be many times higher that these figures. Typically, the number of different donor building block polynucleotides donating/providing a reactant, such as a functional entity, to the synthesis of a library of different scaffolded molecules, will be in the order of at least 100, such as at least 1000, for example at least 10000, such as at least 100000 different donor building block polynucleotides (selected from donor CPNs and/or donor CCPNs).

The hybridisation complex allowing the above-mentioned formation of a scaffolded molecule to take place preferably comprises at least n CPNs, n being an integer of from 2 to 10, preferably from 2 to 8, such as from 2 to 6, for example from 2 to 5, such as from 3 to 10, preferably from 3 to 8, such as from 3 to 6, for example from 3 to 5, and at least n CCPNs, such as at least n+1 CCPNs, for example at least n+2 CCPNs, such as at least n+3 CCPNs, for example at least n+4 CCPNs, such as at least n+5 CCPNs, for example at least n+6 CCPNs, such as at least n+7 CCPNs, for example at least n+8 CCPNs, such as at least n+9 CCPNs, for example at least n+10 CCPNs, such as at least n+11 CCPNs, for example at least n+12 CCPNs, such as at least n+13 CCPNs, for example at least n+14 CCPNs, such as at least n+15 CCPNs.

Covalent bonds between donor CPPNs (or donor CPNs) and their functional entities can be cleaved before, during or after the synthesis of the molecule.

The molecule formed on an acceptor CCPN does not comprise the linker or the polynucleotide part of the acceptor CCPN. Accordingly, the generation of a molecule does not result from a covalent addition of nucleotide(s) to the polynucleotide of the CCPN to which the molecule is linked when the functional entity reactive group reactions have taken place and covalent bonds cleaved between functional entities and donor CCPNs.

Also, in one embodiment the synthesis methods of the present invention do not result in the the formation of a double-stranded polynucleotide molecule in the form of joined or ligated nucleotides of CPNs or CCPNs after the small molecule or polymer has been formed.

Accordingly, the at least one molecule being synthesised by the methods of the invention are distinct from molecules obtained by ligating or joining nucleotide fragments, including double stranded nucleotide fragments.

Furthermore, the methods of the present invention do not involve ribosome mediated translation and prior art methods employing ribosomes for translation purposes are therefore not pertinent to the present invention and are disclaimed as such.

Accordingly, the at least one molecule is generated when, in one embodiment, functional entities on separate complementary connector polynucleotides (CCPNs) are joined by reactions involving functional entity reactive groups. The formation of the molecule is a result of the formation of covalent bonds formed between functional entities constituting the molecule as well as the cleavage of covalent bonds between at least some of the functional entities and the polynucleotide part of the CCPN having donated a particular functional entity, or a part thereof, to the molecule.

The methods of the invention are preferably carried out without cleaving the polynucleotide sequence(s) of CPNs or CCPNs during the synthesis and formation of the molecule.

Accordingly, reactions involving functional entity reactive groups can lead to the formation of a molecule comprising covalently linked functional entities donated by separate CCPNs from which functional entities have been cleaved. The cleavage of the functional entities results in the donor CCPNs not being covalently linked to the molecule. The donation of any single functional entity can occur in a single step or sequentially in one or more steps, and the donation of a plurality of functional entities can occur simultaneously or sequentially in one or more steps.

When reactive groups of a CCPN are located in one embodiment at both (or all) termini of the polynucleotide of a CCPN, functional entity reactive groups of at least some CCPNs participating in the synthesis of the molecule are preferably located only at one of said terminal positions of the polynucleotide. It is such functional entity reactive groups the reaction of which result in the formation of the molecule. However, other reactive groups can be present in the terminal position(s) not occupied by the functional entity comprising functional entity reactive groups. Such reactive groups are different from functional entity reactive groups in so far as these "other" reactive groups do not participate in the synthesis and formation of the molecule.

One example of such "other" reactive groups is e.g. a natural 5'-phosphate group of the polynucleotide of a CCPN comprising a functional entity comprising at least one reactive group at its 3'-terminal end. Another example of a reactive group which is not regarded as a functional entity reactive group is e.g. the natural 3'-hydroxy group of the polynucleotide of a CCPN comprising a functional entity comprising at least one reactive group at its 5'-terminal end.

Accordingly, in one embodiment a functional entity comprising functional entity reactive group(s) is preferably located at one of the terminal end(s) of a CCPN and only functional entity reactive groups are reacted in order to generate a molecule comprising covalently linked functional entities donated by separate CCPNs without said functional entity donation ultimately (i.e. after the molecule has been formed) resulting in CCPNs being covalently linked to each other.

Preferably, at least one functional entity reactive group reaction involving e.g. 2, 3, 4, or more functional entity reactive groups preferably does not result in a CCPN be-

ing joined to other polynucleotides or CCPNs at both the 5'-terminal end and the 3'-terminal end of the polynucleotide of the CCPN at the time the molecule has been generated by covalently linking functional entities donated by separate CCPNs.

Accordingly, there is provided in one embodiment methods wherein at least some CCPNs comprise both functional entity reactive groups and other reactive groups, and wherein reactions at both (or all) terminal positions of the polynucleotide of such CCPNs are not all functional entity reactive group reactions. Only reactive groups the reaction of which results in the formation of the molecule comprising covalently linked functional entities are functional entity reactive groups.

When functional entity reactive groups and other reactive groups are located within the same CCPN, the different kinds of reactive groups will most often be located at different terminal ends of the polynucleotide of the CCPN. Accordingly, functional entity reactive group(s) will generally be separated from other reactive groups of a CCPN by a nucleotide or a nucleobase or a phosphate group.

Preferred aspects of the methods for the synthesis of at least one molecule, or for the synthesis of a plurality of different molecules, are described herein elsewhere.

In one embodiment, the at least one molecule comprising covalently linked functional entities is linked to the polynucleotide part of a complementary connector polynucleotide, but the molecule does not comprise the linker and the polynucleotide part of said complementary connector polynucleotide.

In one embodiment, when the at least one molecule has been formed and covalent bonds created between the functional entities of the molecule, said functional entities are no longer covalently linked to the (donor) CCPNs having donated functional entities or parts thereof to the molecule. The functional entity of a CCPN is preferably attached to a nucleobase by means of a cleavable linker. Such linkers can be cleaved e.g. by acid, base, a chemical agent, light, electromagnetic radiation, an enzyme, or a catalyst.

Accordingly, in one embodiment of the invention, following molecule formation, complementary connector polynucleotides hybridized to connector polynucleotides

are not linked by covalent bonds. Also, in another embodiment, connector polynucleotides (CPNs) hybridized to complementary connector polynucleotides are not linked by covalent bonds. Consequently, such methods are distinct from both ribosome mediated translation of a single template of covalently linked nucleotides and from methods involving nucleotide synthesis and/or ligation as the latter methods result in the formation of ligation products in which nucleotides become covalently linked to each other.

The CCPN polynucleotides can comprise hybridisable nucleotide sequences such as e.g. natural and/or unnatural polynucleotides such as e.g. DNA, RNA, LNA, PNA, and morpholino sequences. The CPN polynucleotides are preferably amplifiable polynucleotides and more preferably polynucleotides comprising DNA and/or RNA. One or more CPNs can be bound to a solic support.

The number or CPNs and/or CCPNs provided for the synthesis of a single molecule can be from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as

from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

Although it is preferred in some embodiments to react at least 3 or more functional entity reactive groups when synthesizing the at least one molecule, in certain other embodiments only 2 reactive groups need to be reacted. The number of reactive groups reacted will depend on the number of functional entities used for the synthesis of the molecule.

When the present invention in one embodiment provides a method for synthesising at least one molecule comprising the steps of

i) providing a plurality of connector polynucleotides each capable of hybridizing to at least 1 complementary connector polynucleotide,

ii) providing a plurality of complementary connector polynucleotides selected from the group consisting of

a) complementary connector polynucleotides comprising at least 1 reactant, such as a functional entity comprising at least 1 reactive group,

b) complementary connector polynucleotides comprising at least 1 reactive group,

c) complementary connector polynucleotides comprising at least 1 spacer region,

iii) hybridizing at least 2 complementary connector polynucleotides to at least 2 connector polynucleotides,

wherein at least 2 of said complementary connector polynucleotides comprise at least 1 reactant, such as a functional entity comprising at least 1 reactive group,

wherein at least 1 of said complementary connector polynucleotides hybridizes to at least 2 connector polynucleotides, and

iv) reacting at least 2 reactants or functional entity reactive groups by reacting at least 1 reactive group of each reactant or functional entity,

wherein the reaction of said reactants or functional entity reactive groups results in the formation of the molecule by reacting the reactive groups of the reactants, or by covalently linking at least 2 functional entities provided by separate complementary connector polynucleotides.

Step iv) can e.g. comprise an embodiment wherein at least 3 reactants or functional entity reactive groups, such as at least 4 reactants or functional entity reactive groups, for example at least 5 reactants or functional entity reactive groups, such as at least 6, such as at least 8, for example at least 10 reactants or functional entity reactive groups, by reacting at least 1 reactive group of each reactant or functional entity.

In one embodiment the method preferably comprises in steps iii) and iv),

iii) hybridizing at least 3 complementary connector polynucleotides to at least 2 connector polynucleotides,

wherein at least 3 of said complementary connector polynucleotides comprise at least 1 reactant, such as a functional entity comprising at least 1 reactive group,

wherein at least 1 of said complementary connector polynucleotides hybridizes to at least 2 connector polynucleotides,

and

iv) reacting at least 3 reactants or functional entity reactive groups by reacting at least 1 reactive group of each reactant or functional entity,

wherein the reaction of said reactants or functional entity reactive groups results in the formation of the molecule by reacting the reactive groups of the reactants, or by covalently linking at least 3 functional entities provided by separate complementary connector polynucleotides.

Step iv) can e.g. comprise an embodiment wherein at least 4 reactants or functional entity reactive groups are reacted, such as at least 5 reactants or functional entity reactive groups are reacted, for example at least 6 reactants or functional entity reactive groups are reacted, such as at least 8 reactants or functional entity reactive groups, such as at least 10, for example at least 12 reactants or functional entity

reactive groups are reacted, by reacting at least 1 reactive group of each reactant or functional entity.

In one embodiment the method preferably comprises in steps iii) and iv),

iii) hybridizing at least 4 complementary connector polynucleotides to at least 2 connector polynucleotides,

wherein at least 4 of said complementary connector polynucleotides comprise at least 1 reactant such as a functional entity comprising at least 1 reactive group,

wherein at least 1 of said complementary connector polynucleotides hybridizes to at least 2 connector polynucleotides,

and

iv) reacting at least 4 reactants or functional entity reactive groups by reacting at least 1 reactive group of each reactant or functional entity,

wherein the reaction of said reactants or functional entity reactive groups results in the formation of the molecule by reacting the reactive groups of the reactants, or by covalently linking at least 4 functional entities provided by separate complementary connector polynucleotides.

Step iv) can e.g. comprise an embodiment wherein at least 5 reactants or functional entity reactive groups are reacted, such as at least 6 reactants or functional entity reactive groups are reacted, for example at least 8 reactants or functional entity reactive groups are reacted, such as at least 10 reactants or functional entity reactive groups are reacted, for example at least 12 reactants or functional entity reactive groups are reacted, such as at least 14 reactants or functional entity reactive groups are reacted, by reacting at least 1 reactive group of each reactant or functional entity.

In one embodiment the method preferably comprises in steps iii) and iv),

iii) hybridizing at least 5 complementary connector polynucleotides to at least 2 connector polynucleotides,

wherein at least 5 of said complementary connector polynucleotides comprise at least 1 reactants, such as a functional entity comprising at least 1 reactive group,

wherein at least 1 of said complementary connector polynucleotides hybridizes to at least 2 connector polynucleotides,

and

iv) reacting at least 5 reactants or functional entity reactive groups by reacting at least 1 reactive group of each reactant or functional entity,

wherein the reaction of said reactants or functional entity reactive groups results in the formation of the molecule by reacting the reactive groups of the reactants, or by covalently linking at least 5 functional entities provided by separate complementary connector polynucleotides.

Step iv) can e.g. comprise an embodiment wherein at least 6 reactants or functional entity reactive groups are reacted, such as at least 7 reactants or functional entity reactive groups are reacted, for example at least 8 reactants or functional entity reactive groups are reacted, such as at least 10 reactants or functional entity reactive groups are reacted, for example at least 12 reactants or functional entity reactive groups are reacted, such as at least 14 reactants or functional entity reactive groups are reacted, for example at least 16 reactants or functional entity reactive groups are reacted, such as at least 18 reactants or functional entity reactive groups are reacted, by reacting at least 1 reactive group of each reactant or functional entity.

The above method of can comprise the further step(s) of hybridizing at least 1 further complementary polynucleotide selected from the group consisting of

a) complementary connector polynucleotides comprising at least 1 functional entity comprising at least 1 reactive group,

b) complementary connector polynucleotides comprising at least 1 reactive group,

c) complementary connector polynucleotides comprising at least 1 spacer region,

to the hybridization complex of step iii), such as to at least 1 connector polynucleotide hybridized to a complementary connector polynucleotide in this complex

and/or the further step(s) of hybridizing at least 1 further connector polynucleotide to the hybridization complex of step iii), such as to at least 1 complementary connector polynucleotide hybridized to a connector polynucleotide in this complex.

The above further step(s) can be repeated as often as required and at least e.g. 2 or 3 times, such as 4 or 5 times, for example 6 or 7 times, such as 8 or 9 times, for example 10 or 11 times, such as 12 or 13 times, for example 14 or 15 times, such as 16 or 17 times, for example 18 or 19 times, such as 20 or 21 times, for example 22 or 23 times, such as 24 or 25 times, for example 26 or 27 times, such as 28 or 29 times, for example 30 or 31 times, such as 32 or 33 times, for example 34 or 35 times, such as 36 or 37 times, for example 38 or 39 times, such as 40 or 41 times, for example 42 or 43 times, such as 44 or 45 times, for example 46 or 47 times, such as 48 or 49 times, for example 50 times.

It is also possible to repeat steps iii) and iv) of the above method at least once, such as 2 or 3 times, such as 4 or 5 times, for example 6 or 7 times, such as 8 or 9 times, for example 10 or 11 times, such as 12 or 13 times, for example 14 or 15 times, such as 16 or 17 times, for example 18 or 19 times, such as 20 or 21 times, for example 22 or 23 times, such as 24 or 25 times, for example 26 or 27 times, such as 28 or 29 times, for example 30 or 31 times, such as 32 or 33 times, for example 34 or 35 times, such as 36 or 37 times, for example 38 or 39 times, such as 40 or 41 times, for example 42 or 43 times, such as 44 or 45 times, for example 46 or 47 times, such as 48 or 49 times, for example 50 times.

In some preferred embodiments, at least n connector polynucleotides and at least n-1 complementary connector polynucleotides are provided, n being an integer preferably of from 3 to 6, and each complementary connector polynucletide hybridizes to at least 2 connector polynucleotides. n can thus be 3 or 4 or 5 or 6. In other embodiments, n can be more than 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

Below is described further embodiments of the methods of the invention for synthesising at least one molecule. The below embodiments are concerned with the provision of different types of hybridisation complexes comprising a plurality of CPNs hybridised to a plurality of CCPNs. The below non-exhaustive examples and embodiments specify some of the possibilities for providing CPNs and CCPNs and forming hybridisation complexes comprising a plurality of CPNs hybridised to a plurality of CCPNs. The examples are illustrated in Fig. 4 herein. It will be understood that all or only some of the CPNs and CCPNs provided can comprise a polynucleotide part linked to a reactant (capital letters in Fig. 4). For all of the below embodiments, the at least one molecule can be generated by reacting reactants positioned on separate CPNs and/or separate CCPNs prior to the formation of the at least one molecule.

In one embodiment, at least n connector polynucleotides and at least n complementary connector polynucleotides are provided, n being an integer of preferably from 3 to 6, and at least n-1 complementary connector polynucletide hybridize to at least 2 connector polynucleotides. There is also provided a method wherein n complementary connector polynucletide hybridize to at least 2 connector polynucleotides. n can thus be 3 or 4 or 5 or 6. In other embodiments, n can be more than 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example

41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

In yet another embodiment, at least n connector polynucleotides and at least n+1 complementary connector polynucleotides are provided, n being an integer of preferably from 3 to 6, and at least n-1 complementary connector polynucleotide hybridize to at least 2 connector polynucleotides. It is alos possible that n complementary connector polynucletide hybridize to at least 2 connector polynucleotides. n can thus be 3 or 4 or 5 or 6. In other embodiments, n can be more than 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50. There is also provided a method wherein n complementary connector polynucletide hybridize to at least 2 connector polynucleotides.

In a still further embodiment, at least n connector polynucleotides and at least n+2 complementary connector polynucleotides are provided, n being an integer of preferably from 3 to 6, and at least n-1 complementary connector polynucletide hybridize to at least 2 connector polynucleotides. It is also possible for n complementary connector polynucletide to hybridize to at least 2 connector polynucleotides. n can thus be 3 or 4 or 5 or 6. In other embodiments, n can be more than 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

In yet another embodiment, at least n connector polynucleotides and at least n+3 complementary connector polynucleotides are provided, n being an integer of preferably from 3 to 6, and at least n-1 complementary connector polynucletide hybridize to at least 2 connector polynucleotides. It is also possible for n complementary connector polynucletide to hybridize to at least 2 connector polynucleotides. n can

thus be 3 or 4 or 5 or 6. In other embodiments, n can be more than 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

In a further embodiment at least n connector polynucleotides and at least n+4 complementary connector polynucleotides are provided, n being an integer of from preferably 3 to 6, and at least n-1 complementary connector polynucletide hybridize to at least 2 connector polynucleotides. It is also possible for n complementary connector polynucletide to hybridize to at least 2 connector polynucleotides. n can thus be 3 or 4 or 5 or 6. In other embodiments, n can be more than 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

In still further embodiments, there is provided methods wherein n connector polynucleotides and at least n+5, such as at least n+6, for example n+7, such as at least n+8, for example n+9, such as at least n+10, for example n+11, such as at least n+12, for example at least n+13, such as n+14, for example at least n+15, such as n+16, for example at least n+17, such as n+18, for example at least n+19, such as n+20, for example at least n+21, such as at least n+22, for example n+23, such as at least n+24, for example n+25 complementary connector polynucleotides are provided, n being an integer of preferably from 3 to 6, and at least n-1 or n complementary connector polynucletide hybridize to at least 2 connector polynucleotides. n can also be more than 6, such as e.g. such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

In all of the above-mentioned methods it is furthermore possible for any plurality of complementary connector polynucleotides to hybridise to a single connector polynucleotide of the supramolecular complex. Any plurality can be e.g., but not limited to, 2 or 3, for example 4 or 5 or 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

More than one single connector polynucleotide can be hybridized to the above plurality of complementary connector polynucleotides, such as 2 single connector polynucleotides, for example 3 or 4 single connector polynucleotides, such as 5 or 6 single connector polynucleotides, for example 7 or 8 single connector polynucleotides, such as 9 or 10 single connector polynucleotides, for example 11 or 12 single connector polynucleotides, such as 13 or 14 single connector polynucleotides, for example 15 or 16 single connector polynucleotides, such as 17 or 18 single connector polynucleotides, for example 19 or 20 single connector polynucleotides.

The plurality of connenctor polynucleotides provided can comprise linear and/or branched connector polynucleotides. In one embodiment , the plurality of connenctor polynucleotides comprise at least n branched connector polynucleotides and at least n complementary connector polynucleotides, n being an integer of preferably from 2 to 6, and wherein at least n-1 complementary connector polynucletide hybridize to at least 2 branched connector polynucleotides. In other embodiments there is provided at least n+1 complementary connector polynucleotides. Also, it is possible for at least n such as n+1 complementary connector polynucleotides to hybridize to at least 2 branched connector polynucleotides. n can thus be 3 or 4 or 5 or 6. In other embodiments, n can be more than 6, such as 7 or 8, for example 9 or 10, such as 11 or 12, for example 13 or 14, such as 15 or 16, for example 17 or 18, such as 19 or 20, for example 21 or 22, such as 23 or 24, for example 25 or 26, such as 27 or 28, for example 29 or 30, such as 31 or 32, for example 33 or 34, such as 35 or 36, for example 37 or 38, such as 39 or 40, for example 41 or 42, such as 43 or 44, for example 45 or 46, such as 47 or 48, for example 49 or 50.

In one embodiment, a molecule of the invention is formed when functional entities are transferred from donor complementary connector polynucleotides to an acceptor complementary connector polynucleotide. Accordingly, one or more reactive group(s) of at least 1 functional entity of a complementary connector polynucleotide react with one or more reactive group(s) of at least 1 functional entity of at least 1 other complementary connector polynucleotide. The at least 1 functional entity preferably comprise from 1 to 6 reactive groups, such as e.g. 2 or 3 or 4 or 5 reactive groups.

In one preferred embodiment, at least 3 reactive groups of at least 1 functional entity react with at least 1 reactive group of at least 3 other functional entities..The molecule can ultimately be generated on an acceptor complementary connector polynucleotide by covalently linking functional entities, or a part thereof, donated by one or more individual complementary connector polynucleotides (CCPNs) each comprising at least one functional entity, such as 2 or 3 CCPNs, for example 4 or 5 CCPNs, such as 6 or 7 CCPNs, for example 8 or 9 CCPNs, such as 10 or 11 CCPNs, for example 12 or 13 CCPNs, such as 14 or 15 CCPNs, for example 16 or 17 CCPNs, such as 18 or 19 CCPNs, for example 20 or 21 CCPNs, such as 22 or 23 CCPNs, for example 24 or 25 CCPNs.

The plurality of complementary connector polynucleotides preferably comprise at least 2 complementary connector polynucleotides (CCPNs) which are non-identical, such as 10 CCPNs, for example 50 CCPNs, such as 1000 CCPNs, for example 10000 CCPNs, such as 100000 CCPNs which are non-identical.

In one embodiment there is provided a method wherein said plurality of complementary connector polynucleotides comprise at least 2 branched complementary connector polynucleotides.

The plurality of connector polynucleotides preferably comprise connector polynucleotides comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 400, such as 300, for example 200, such as 100, for example 50, such as 40, for example 30. The plurality of connector polynucleotides can further comprise connector polynucleotides comprising at least 1 branching point con-

necting at least three polynucleotide fragments comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 400, such as 300, for example 200, such as 100, for example 50, such as 40, for example 30.

In some embodiments of the invention connector polynucleotides can be selected from the group consisting of

a) connector polynucleotides comprising at least 1 functional entity comprising at least 1 reactive group,

b) connector polynucleotides comprising at least 1 reactive group,

c) connector polynucleotides comprising at least 1 spacer region,

The plurality of complementary connector polynucleotides can comprise polynucleotides comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 400, such as 300, for example 200, such as 100, for example 50, such as 40, for example 30. The plurality of complementary connector polynucleotides can further comprise polynucleotides comprising at least 1 branching point connecting at least three polynucleotide fragments comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 400, such as 300, for example 200, such as 100, for example 50, such as 40, for example 30.

In another aspect of the invention there is provided a method for synthesising a plurality of different molecules, said method comprising the steps of performing any of the methods described herein above for each different molecule being synthesised.

Further steps in the method for synthesising a plurality of different molecules are provided herein below. One further step comprises selecting molecules having desirable characteristics, wherein the selection employs a predetermined assaying procedure.

Another further step is amplifying at least part of the individual connector polynucleotides used for the synthesis of a selected molecule. Yet another further step is

contacting a population of said amplified connector polynucleotides, or fragments thereof, with a plurality of complementary connector polynucleotides.

It is also possible to perform an additional synthesis round by carrying out the steps of the method using a population of said amplified connector polynucleotides or a population of said amplified connector polynucleotide fragments.

A still further step is characterised by performing a ligation of individual CPNs or individual CCPNs, optionally preceded by a polynucleotide extension reaction for extending gaps and e.g. duplex polynucleotides further comprising a single stranded part selected from the group consisting of a non-hybridizing part of a connector polynucleotide and a non-hybridizing part of a complementary connector polynucleotide.

Further steps pertaining to this method are

a) digesting said ligated and optionally extended duplex polynucleotides,

b) displacing the duplex polynucleotides, thereby generating single polynucleotide strands of extended connector polynucleotides and extended complementary connector polynucleotides, and

c) contacting digested, extended and displaced connector polynucleotides with a plurality of complementary connector polynucleotides, after which it is possible to

performing an additional synthesis round by carrying out the steps of the method using a population of said ligated (and optionally extended), digested and displaced connector polynucleotides.

The invention also pertains to bifunctional molecules comprising a molecule part and a hybridisation complex part comprising a plurality of hybridised building block polynucleotides. The molecules capable of being synthesised by the present invention (i.e. the molecule part of bifunctional molecules) are disclosed in detail

herein below. It will be understood that the invention also pertains to bifunctional molecules comprises such molecules.

Molecules capable of being synthesised by the methods of the present invention include, but is not limited to molecules comprising a linear sequence of functional entities and branched molecules comprising a branched sequence of functional entities. Molecules comprising a cyclic sequence of functional entities can also be provided.

Yet another example of a molecule capable of being synthesised is an oligomer or a polymer comprising at least one repetitive sequence of functional entities. In one embodiment, the sequence of at least three functional entities is preferably repeated at least twice in the molecule, in another embodiment any sequence of at least three functional entities in the molecule occurs only once.

Preferred molecules comprise or essentially consists of amino acids selected from the group consisting of α-amino acids, β-amino acids, γ-amino acids, ω-amino acids, natural amino acid residues, monosubstituted α-amino acids, disubstituted α-amino acids, monosubstituted β-amino acids, disubstituted β-amino acids, trisubstituted β-amino acids, and tetrasubstituted β-amino acids.

The backbone structure of said β-amino acids preferably comprises or essentially consists of a cyclohexane-backbone and/or a cyclopentane-backbone.

Other preferred classes of molecules are molecule comprising or essentially consisting of vinylogous amino acids, and molecule comprises or essentially consists of N-substituted glycines.

Further preferred molecules comprise or essentially consist of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptide, polysulfonamide, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-

substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinone, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, inlcuding any combination thereof.

Yet further preferred molecules are those comprising a scaffold structure comprising a plurality of covalently linked functional entities selected from the group consisting of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptides, polysulfonamides, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, polythioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinones, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, and wherein the plurality of functional entities is preferably from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as

from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

Molecular weights of the molecules to be synthesised in accordance with the present invention are preferably "small molecules", i.e. molecules preferably having a molecular weight (MW) of less than 10000 Daltons, such as less than 8000 Daltons, for example less than 6000 Daltons, such as less than 5000 Daltons, for example less than 4000 Daltons, for example less than 3500 Daltons, such as less than 3000 Daltons, for example less than 2500 Daltons, for example less than 2000 Daltons, such as less than 1800 Daltons, for example less than 1600 Daltons, for example less than 1400 Daltons, such as less than 1200 Daltons, for example less than 1000 Daltons.

The functional entities of the above molecules can be linked by a chemical bond selected from the group of chemical bonds consisting of peptide bonds, sulfonamide bonds, ester bonds, saccharide bonds, carbamate bonds, carbonate bonds, urea bonds, phosphonate bonds,urethane bonds, azatide bonds, peptoid bonds, ether bonds, ethoxy bonds, thioether bonds, single carbon bonds, double carbon bonds, triple carbon bonds, disulfide bonds, sulfide bonds, phosphodiester bonds, oxime bonds, imine bonds, imide bonds, including any combination thereof.

In one embodiment the chemical bond linking at least some of the functional entities of the molecule is preferably formed by a reaction of a nucleophile group of a first functional entity with an ester or thioester of another functional entity. The linker of the functional entity bearing the thioester group is preferably cleaved simultaneously with the formation of the bond resulting in a transfer of the functional entity or a part thereof to the nucleophilic functional entity. The nucleophile group is preferably selected from $-NH_2$, H_2NHN-, HOHN-, $H_2N-C(O)-NH-$.

The backbone structure of a molecule synthesised by the methods of the present invention can comprises or essentially consists of one or more molecular group(s) selected from -NHN(R)CO- ; -NHB(R)CO- ; -NHC(RR')CO- ; -NHC(=CHR)CO- ; $-NHC_6H_4$ CO-; $-NHCH_2$ CHRCO-; $-NHCHRCH_2$ CO- ; $-COCH_2-$; -COS- ; -CONR- ; -COO- ; -CSNH- ; $-CH_2$ NH- ; $-CH_2CH_2-$; $-CH_2$ S- ; $-CH_2$ SO- ; $-CH_2SO_2-$; $-CH(CH_3)S-$; -CH=CH- ; -NHCO- ; -NHCONH- ; -CONHO- ; $-C(=CH_2)CH_2-$; $-PO_2^-NH-$; $-PO_2^-CH_2-$; $-PO_2^-CH_2N^+-$; $-SO_2NH^-$- ; and lactams.

In accordance with the present invention it is possible to generate a composition comprising a plurality of more than or about 10^3 different molecules, such as more than or about 10^4 different molecules, for example more than or about 10^5 different molecules, such as more than or about 10^6 different molecules, for example more than or about 10^7 different molecules, such as more than or about 10^8 different molecules, for example more than or about 10^9 different molecules, such as more than or about 10^{10} different molecules, for example more than or about 10^{11} different molecules, such as more than or about 10^{12} different molecules, for example more than or about 10^{13} different molecules, such as more than or about 10^{14} different molecules, for example more than or about 10^{15} different molecules, such as more than or about 10^{16} different molecules, for example more than or about 10^{17} different molecules, such as more than or about 10^{18} different molecules.

The molecules can be targeted to a potential binding partner while still bound to a CCPN or a CPN of a bifunctional molecule, or the molecules can be cleaved from the CPPN to which they are bound following their synthesis. When targeted to a potential binding partner, the present invention also pertains to complexes further comprising a binding partner having an affinity for the molecule. Such binding partners can be e.g. any another molecule selected from the group consisting of DNA, RNA, antibody, peptide, or protein, or derivatives thereof.

Methods for the synthesis and efficient screening of molecules is described herein above. The below sections describe in further detail selected embodiments and different modes for carrying out the present invention.

The methods of the present invention allows molecules to be formed through the reaction of a plurality of reactants, such as e.g. reactions involving the formation of bonds between functional entities *i.e.* chemical moieties, by the reaction of functional entity reactive groups. The present invention describes the use of connector polynucleotides (CPN's) to bring functional entities in proximity, whereby such bond formations are made possible, leading to the synthesis of molecules such as e.g. small molecules and polymers.

In the present invention, the individual chemical moieties/functional entities may be carried by oligonucleotides (CCPN's) capable of annealing to said CPN's. The combination and reaction of functional entity reactive groups carried by such comple-

mentary connectors polynucleotides, will lead to formation of molecules via complexation to CPN's.

Each CPN may bring two or more CCPN's in proximity, whereby reactions between functional groups on these CCPN's are made more likely to occur. Functional entity reactive groups/reactive moieties/functional groups may be activated scaffolds or activated substituent like moieties etc. Some CCPN's only anneal to one CPN other CCPN's may anneal to two CPN's. In one embodiment of the present invention, a CCPN anneals to a CPN, which CPN allows the annealing of one further CCPN. This second CCPN may then allow the annealing of a second CPN, which may allow annealing of further CCPN's and so forth (See e.g. figure 22). Hybridization of multiple CCPN's and CPN's may be either sequentially or simultaneously in either one or multiple tubes. As such all CCPN's and CPN's may be added at once. Alternatively, they may be added sequentially, *i.e.* e.g. first a set of CPN's, then a set of CCPN's followed by a new set of CPN's or visa versa. In this sequential setting a handling control of CCPN/CPN-complex selfassembly is achieved. In another embodiment, a set of CCPN's forms complexes A^1-A^n with a set of CPN's in one separate compartment e.g. a tube. In other compartments, other sets of CCPN's forms complexes B^1-B^n with a set of CPN's etc. These separately formed complexes may be combined and form further new complexes, either directly or through further addition of CCPN's or CPN's. This illustrates still another way of a handling control of CCPN/CPN-complex selfassembly.

The present invention may be used in the formation of a library of compounds. Each member of the library is assembled by the use of a number of CCPN's, which number may be the same or different for different molecules. This will allow the formation of a mixed library of molecules assembled from 2 to n chemical moieties/fragments/functional entities or parts thereof.

If such a library, e.g. contains molecules assembled from 1-7 functional entities/chemical moieties and 100 different functional entity/moiety types exists, the library would theoretically be a mixture of more than 100^7 molecules. See Figure 3.

In one setting, a CCPN may specify for the annealing of a specific type of CPN, a CPN which will specify the annealing of a further specific second CCPN, which functional entity reactive groups are capable of reacting with the functional entity reactive groups of CCPN one. In this setting each CCPN will therefore specify, which CCPN

it interacts with via the CPN sequence, *i.e.* which reaction partner(s) they accept/prefer.

Some CCPN's carrying scaffolds may contain a certain set of functional groups. Other CCPN's carry scaffolds with another set of functional groups and still, each scaffold carrying CCPN may be combined with other CCPN's, which functional entity reactive groups can react with exactly that scaffold in the presence of a number of other types of CCPN's, including e.g. CCPN's which could have reacted but were not allowed to react. Further details are described below. This control of correct/accepted combinations of functional entity reactive groups will allow the formation of a mixed library of highly branched, semi-branched and linear molecules.

The CCPN cross talk may also be used to control the properties of library members. E.g. CCPN's carrying large functional entities may only call for CCPN's carrying small functional entities or CCPN's carrying hydrophilic entities may call for CCPN's carrying hydrophilic functional entities or lipophilic functional entities depending on design.

As the chemistries applicable, will be increased by the fact, that CCPN's themselves ensure correct/accepted functional entity reaction partners, a much higher number of scaffolds will become easily available and may co-exist. E.g., it may be that derivatization of one scaffold can only be performed through the use of one specific set of transformation, whereas another scaffold may need another set of transformations. Different reactions and different CCPN's will therefore be needed for derivatization of each of these scaffolds. This is made possible by the present invention. See further details below.

As the total number of theoretically synthesizable molecules may exceed the number of actually synthesized molecules, which can be present in a given tube, shuffling becomes important to ensure a maximum of tested CCPN combinations. If e.g. 10^{17} is considered as a potential maximum number of different molecules present in a given reaction tube, then by using 1.000 different CCPN's and allowing formation of molecules assembled from the functional entities of 6 CCPN's, this number will be exceeded. Selection ensures that appropriate CPN's will survive, and shuffling will ensure that the number of combinations tested will be maximized.

In one embodiment of the present invention, a CPN-sequence is designed so as to anneal to one specific CCPN-sequence. This gives a one-to-one relationship be-

tween the functional entity descriptor (e.g. a polynucleotide based codon) and encoded functional entity. However, the same effect, a specific functional entity is encoded by specific CPNs and CCPNs, can be obtained by having a set of CPN-sequences that anneal to a set of CCPN-sequences. This would then require that identical functional entities are carried by all the CPNs or CCPNs of a set.

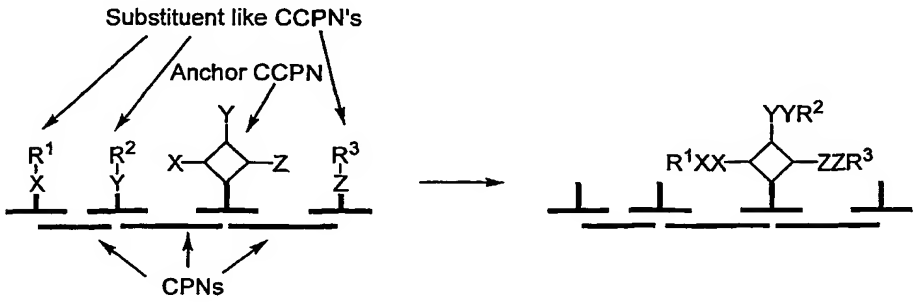
This kind of "codon-randomization" is sometimes advantageous, for example when CPN-sequences and CCPN-sequences are designed so as to allow an expansion of the library size at a later stage. If the coding region of e.g. a CPN is 3 nucleotides (providing 64 different codons), but only 16 different functional entities have been prepared, then the CCPNs may be grouped into 16 groups, for example where the first of the three nucleotide positions is randomized (i.e. 4 different CCPN-sequences carry the same functinal entity). A pseudo-one-to-one relationship is thus preserved, since the identity of the encoded functional entity can be unambigously identified by identification of the CPN (or CCPN) involved.

Sometimes scrambling, i.e. one CPN or CCPN sequence specifying more than one functional entity, is advantagous. Likewise, under certain conditions it is advantagous to have one CPN or CCPN specify more than one functional entity. This will, however, not lead to a one-to-one or a pseudo-one-to-one relationship. But may be advantagous, for example in cases where the recovered (isolated) entity from a selection can be identified through characterization of for example its mass (rather than its attached polynucleotide complex), as this will sample a larger chemistry space.

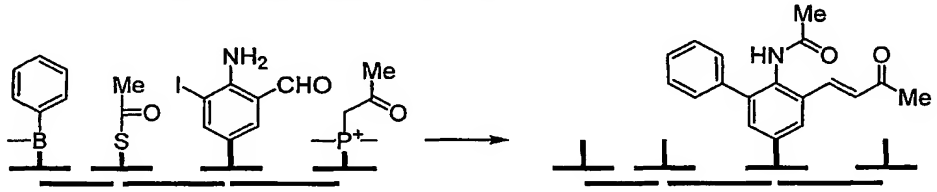
The present invention may use short oligonucleotides, which are easily available in high purity.

In the assembly of a molecule, individual CCPN's are connected via CPN's. The functional group composition of each functional entity on the CCPN, determines the shape of the final molecule. Highly branched molecules may as such be assembled by transfer (or cross linkage followed by (linker) cleavage) of functional entities from multiple mono-functionalized functional entities (*i.e.* comprising one function entity reactive group) of CCPN's (e.g. substituent like) to multi-functionalized functional en-

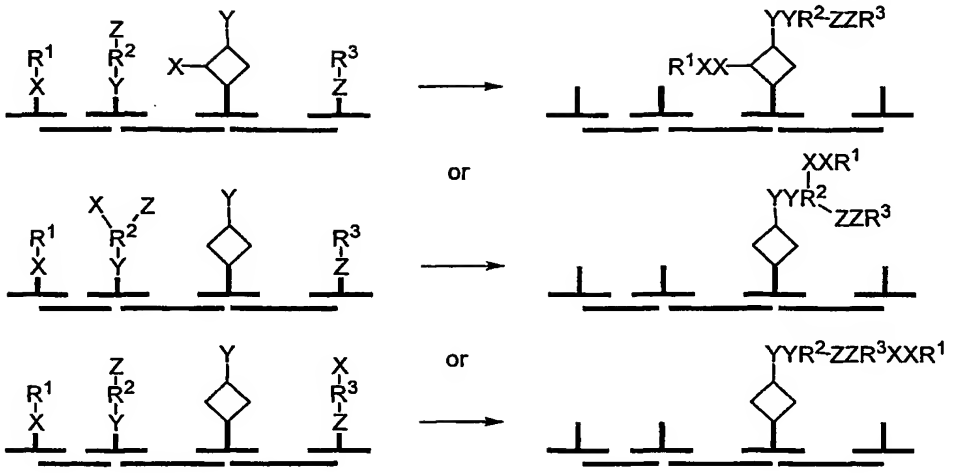
tities (*i.e.* comprising multiple functional entity reactive groups) of CCPN's (e.g. scaffolds/anchor like). Which transfer may be conducted in one or more steps. E.g.:



where X, Y and Z denotes functional entity reactive groups capable of reacting with each other, e.g. an amine reacting with an acylating CCPN etc., and R denotes a substituent such e.g. methyl, phenyl etc. E.g.:

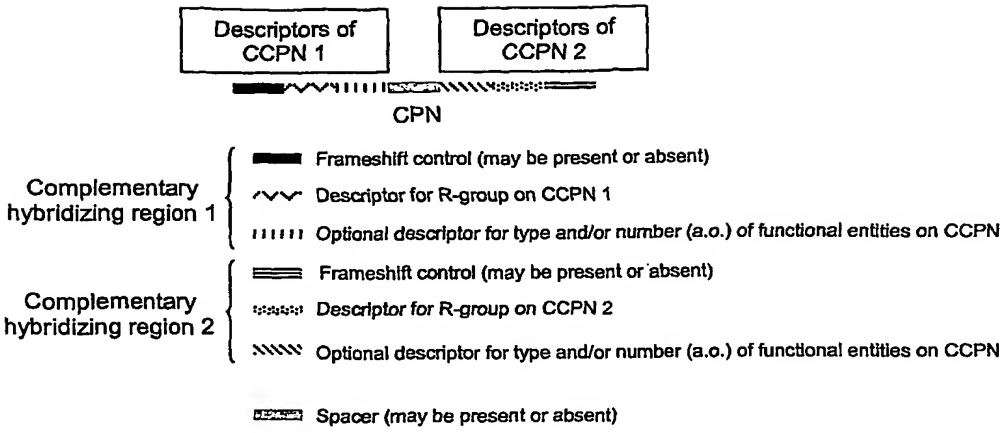


Linear molecules on the other hand, demands that the functional entity of the anchor/scaffold like CCPN contains less activated functionalization (*i.e.* fewer functional entity reactive groups), and furthermore that the functional entity reactive groups of substituent like CCPN's reacts with each other. E.g.:



However, in the formation of a library which both contains a mixture of highly branched, less branched and linear molecules, it is important to control, that the number and type of functional groups capable of reacting with each other match.

The use of a plurality of CPN's solves this issue, by allowing only specific combinations of CCPN's in the encoding of each molecule. Each CPN thereby ensures a specific match between the number and type of needed reactions. The simplest CPN, for annealing two CCPN's could be composed like:

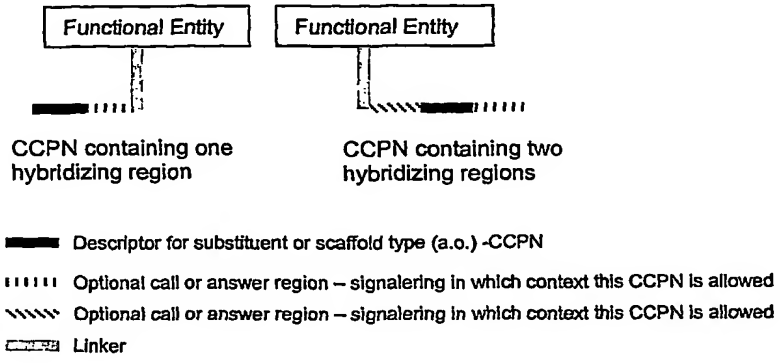


The exact position of domain types may be varied as appropriate.

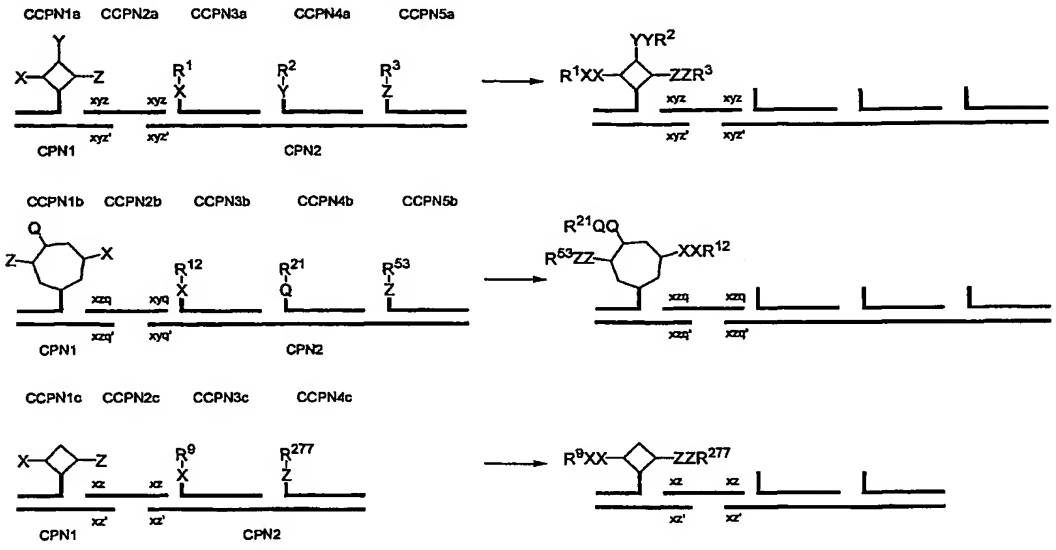
In the formation of a molecule, a plurality of CPN's is used. In the generation of a library of molecules, each molecule will be assembled through the use of individual combinations of CPN's. A library of molecules may be prepared as individually separated compounds or as a mixture of compounds.

Each set of CPN's will contain variable polynucleotide regions in the domains for the descriptors for R-groups, and each of these variable polynucleotide regions may be combined with different combinations of CCPN annealing capabilities.

Similarly, may CCPN's, in their hybridizing domains specify/signal the need for specific reaction partners.

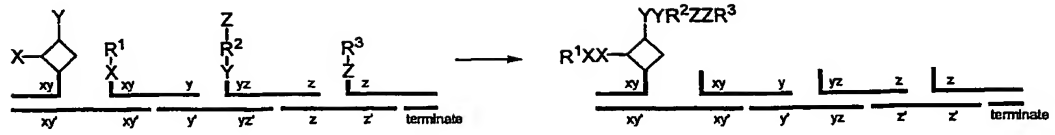


In a very simple setting, the scaffold carrying CCPN1's signals the need for one specific set (type and number) of substituent like CCPN's. E.g.,



Each scaffold will thereby be derivatized appropriately, according to the needed types and numbers of reaction partners. The CCPN1, *i.e.* the scaffold like/anchor CCPN signals the need for a set of substituent CCPN's via annealing to an appropriate CPN1. This CPN1 calls for another CCPN2, which in this example corresponds to a spacer. The CCPN2 carry on the call for the appropriate CPN2, carrying the appropriate substituent like CCPN's via annealing of these to that CPN2. In this example, the substituent like CCPN's can only be brought in proximity to the appropriate scaffold/anchor CCPN and thereby allowed to react, if the chemistries fit, which is signaled through CCPN cross talk via CPN's. The complexes of CPN's and CCPN's described in the present invention may optionally contain single stranded regions.

Another extreme would be the setting where each individual CCPN signals its own need for reaction partners. With mono-directional scaffold derivatization one design/embodiment could be like the following:



The anchor/scaffold CCPN carries two functional groups X and Y in the functional entity. It therefore signals the call for X and Y partners. The first substituent like CCPN carries only a functional group X and answers by signaling this, as it furthermore calls for a substituent like CCPN carrying functional entity reactive group Y. These "calls/answers" are mediated via the CPN, without which these two CCPN's would not be brought in proximity and allowed to react.

The second substituent like CCPN answers the call for a functional entity reactive group Y, but since this CCPN also carries a functional entity reactive group Z, it calls for that. The third substituent like CCPN answers the call for a functional entity reactive group Z, but does not call for further CCPN's. A terminator CPN may optionally anneal to the fourth complementary connector. As can be seen, the answer signal may optionally also contains information about, what exactly this CCPN further calls for. In other words, the call signal may be answered by the availability of functional entity reactive groups as well as the one which are further called for.

The CPN's may be amplified at some step in the process or optionally be ligated to yield a one length polynucleotide, which may also be amplified and optionally further manipulated.

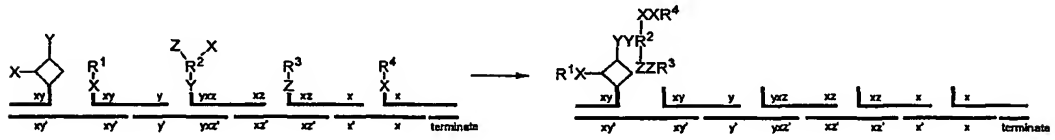
After e.g. selection/enrichment of the CPN/CCPN/small molecule complexes with desired characteristics (e.g. binding affinity for a protein target), the CPNs and CCPNs recovered may be amplified before characterization or a further round of selection, by any of several means:

1. Oligonucleotide primers that anneal to the terminal regions of the CPNs and CCPNs are added, and a PCR-reaction performed. This leads to the amplification of the oligonucleotide portion of all the individual CPNs and CCPNs. When the CPNs and CCPNs carry functional entities, these functional entities can be coupled to one of the two primers that anneal to a CPN or CCPN. This will lead to the amplification of this CPN or CCPN with its functional entity.

2. A PCR reaction may be performed without the addition of primers. After a number of PCR cycles (e.g. 20-30 cycles), external primers can be added. This will result in the generation of longer DNA-molecules, spanning the length of the quasirandom complexes. If the CPNs and CCPNs have been appropriately designed, cleavage by restriction nucleases can regenerate the CPNs and CCPNs, ready for a new round of quasirandom complex and small molecule formation.

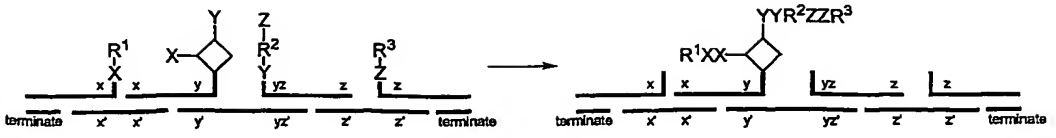
3. The CPNs or the CCPNs may be ligated together, e.g. using a DNA ligase. This will result in the generation of longer DNA-molecules spanning the length of the quasirandom complexes. If the CPNs and CCPNs have been appropriately designed, cleavage by restriction nucleases can regenerate the CPNs and CCPNs, ready for a new round of quasirandom complex and small molecule formation.

The same scaffold as described above could end up as a more branched molecule in another combination of CCPN's, e.g.:



The difference between the two examples being, that the second substituent like CCPN in this setting was different, but still answered the call for a Y substitutent like CCPN from the first X substitutent like CCPN. Another difference being, that this CCPN makes its own call for both a Z and an X functional entity reactive group carrying subsitutent like CCPN. In this example scrambling may occur due to the fact that the calls allowed two different X functional entity reactive group carrying substituent like CCPN's to anneal.

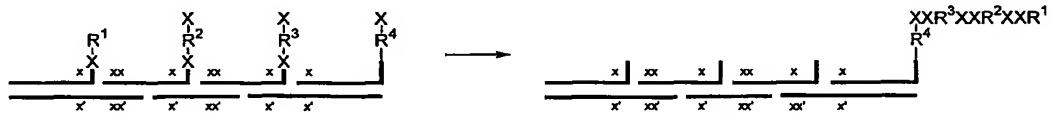
In another setting, one may use bi-directional scaffold derivatization, such as e.g.:



In this setting the scaffold/anchor CCPN contains two call regions, one at each terminus. Such a setting may be useful in a multiple CCPN settings, as substituent CCPN's are brought in higher proximity to the anchor CCPN.

In between settings of the above is also possible, *i.e.* a combination where some CPN's hybridizes multiple CCPN's, whereas other CPN's only hybridizes one or two CCPN's.

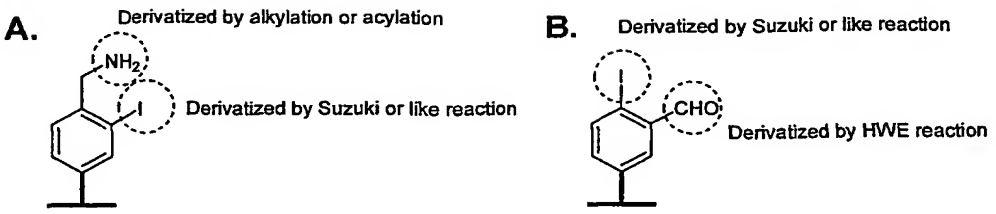
The following example illustrates one example of a setup for the formation of a linear molecule.



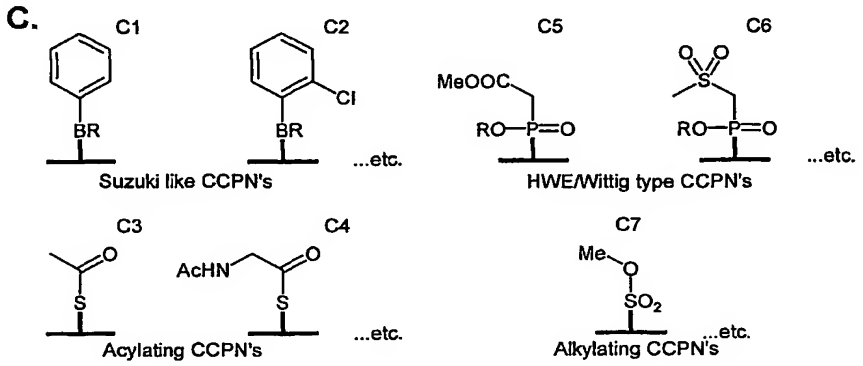
In this setting the first CCPN signals the call to undergo an "x"-reaction, which is answered by CCPN number two, which further signals the call to undergo an "x"-reaction etc. The fourth CCPN does not make any further calls.

The following section describes how hybridization regions may be designed for CCPN's and CPN's. Each region may specify, the needed types/numbers of reaction partners.

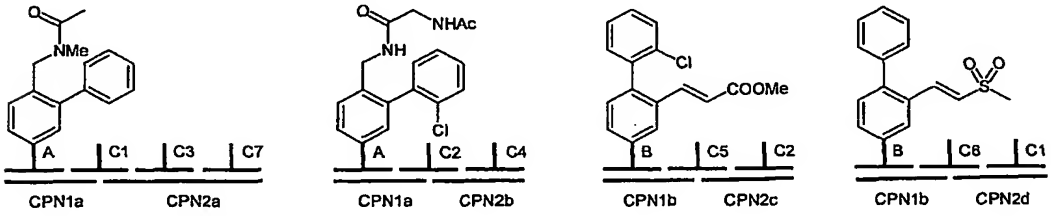
The following simple example illustrates one design. Two different scaffold like CCPN's A and B demands different types of functional entity reaction group chemistries.



They are then to be combined with a set of substituent like CCPN's as illustrated e.g. C1-C7.



In the very simple setting, the scaffold like CCPN's calls for all the substituents needed, where such substituents are hybridized to e.g. the same CPN, *i.e.* only two CPN's are used. The four synthesized molecules below illustrate some of the products found in the library.



CPN type1a anneals the scaffold type A and calls for (can only combine with) CCPN's carrying functional entity reactive groups capable of undergoing acylation and/or alkylation and furthermore a CCPN carrying functional entity reactive groups capable of undergoing a Suzuki reaction. This ensures e.g. that CCPN's carrying

functional entity reactive groups capable of undergoing e.g. HWE reaction will not be combined with scaffold like CCPN type A.

CPN type 2a carries three CCPN's with functional entity reactive groups capable of undergoing acylation, alkylation and Suzuki type reactions.

CPN type 2b carries only two CCPN's with functional entity reactive groups capable of undergoing acylation and Suzuki type reactions.

CPN type 2a thereby allows further branching, whereas CPN type 2b does not.

CPN type 1b anneals the scaffold type B and calls for (can only combine with) CCPN's carrying functional entity reactive groups capable of undergoing HWE/Wittig reaction and furthermore a CCPN carrying functional entity reactive groups capable of undergoing a Suzuki reaction. This ensures e.g. that CCPN's carrying functional entity reactive groups capable of undergoing e.g. acylation reaction will not be combined with scaffold like CCPN type B.

If all four bases are used in the variable regions of CCPN's a total and e.g. 256 different scaffolds type A, 256 different scaffolds type B, 256 different acylating CCPN's, 256 different alkylating CCPN's, 256 Suzuki type CCPN's and 256 different HWE/Wittig type CCPN's could be used. The following sequences for polynucleotide sequences could be one design to illustrate the principle (wherein N denotes a random nucleobase, preferably selected from G, A, C, T, U):

Scaffold like CCPN's type A's: 3'-GCGCNNNNGGCG-5'

One specific scaffold e.g. the one illustrated above could e.g. have the specific sequence: 3'-GCGCATTAGGCG-5'.

Another scaffold type A, demanding the same chemistries but having another skeleton could have the specific sequence: 3'-GCGCTTAAGGCG-5' etc.

Scaffold like CCPN's type B's: 3'-AATTNNNNTAAT-5'

One specific scaffold e.g. the one illustrated above could e.g. have the specific sequence: 3'-AATTGCCGTAAT-5'.

Another scaffold type A, demanding the same chemistries but having another skeleton could have the specific sequence: 3'-AATTCGGGTAAT-5' etc.

Suzuki type CCPN's: 3'-TTTTTGAGANNNNAAGGTTTTT-5'

One specific Suzuki type CCPN e.g. C1 illustrated above could e.g. have the specific sequence: 3'-TTTTTGAGATTCCAAGGTTTTT-5'. Another Suzuki type CCPN could e.g. have the sequence 3'-TTTTTGAGACTTCAAGGTTTTT-5'.

Acylation type CCPN's: 3'-GTTGNNNNTTGG-5'

Alkylation type CCPN's: 3'-AACCNNNNACCA-5'

HWE/Wittig type CCPN's: 3'-TTCCNNNNCTCT-5'

CPN type 1a sequences: 3'-NNNNTCTCAAAAACGCCNNNNGCGC-5'
One specific type of these would be 3'-GGAATCTCAAAAACGCCTAATGCGC-5' this CPN would allow the hybridization of CCPN type A and CCPN type C1.
Another specific sequence would allow the hybridization of e.g. C2 instead of C1 but not C3-C7 etc.

In some settings single stranded regions may be applied to increase flexibility of the complex. This may be implemented by increasing e.g. the number of A nucleobases from 5 nucleobases to 7 or 10 or what is found appropriate.

CPN type 2a sequences: 3'-TGGTNNNNGGTTCCAANNNNCAACAAAAACCTT-5'

CPN type 2b sequences: 3'-CCAANNNNCAACAAAAACCTT-5'

Sequences for CPN type 1b, 2c and 2d are designed similarly to allow hybridization of CCPN's carrying functional entity reactive groups capable of undergoing HWE reactions rather than acylating and/or alkylating reactions.

If the number of potential combination is to be maximally increased a high number of CPN's may be used and each CCPN may then make use of "cross talk".

In such a setting, the reactions used may be 1. acylations (Ac), 2. alkylations (Al) 3. Cross coupling/Suzuki and like reactions (C) and 4. HWE/Wittig type reactions (W). Each reaction demands a donor and an acceptor, where donor denotes a functional entity reactive group, which upon reaction leads to transfer of the functional entity or a part thereof of that CCPN. Transfer may be directly in one step or sequentially through cross linkage followed by cleavage. An acceptor denotes a functional entity reactive group, which upon reaction accepts the transfer of a functional entity or part thereof from another CCPN.

When designing CCPN hybridization regions, one may bias the library towards specific properties, e.g. if selection is used to identify drug candidates in the library, it is in most cases not appropriate to have aromatic amines presented due to their potential toxic properties, whereas aliphatic amines are in general acceptable. CCPN's carrying aromatic amines may therefore specifically signal the need to be partnered, with a CCPN carrying a functional entity reactive group capable of undergoing acylation reactions and optionally allow a CCPN carrying a functional entity reactive group capable of undergoing alkylating reactions, whereas aliphatic amines may be partnered with both CCPN's carrying functional entity reactive groups capable of undergoing acylation and alkylation reactions. Aromatic hydroxyl groups, on the other hand, should not be acylated due to the generation of another acylating specie, which will generally not be acceptable as drug candidate. Aromatic hydroxyl groups should therefore only be alkylated. Such demands may be entered into hybridization region for a specific CCPN.

If all four reaction types were to be used in one library generation, then the hybridization region of each CCPN could specify, which one of the reaction types, mentioned above, are needed (denoted by "*"), allowed (denoted by "+") and forbidden (denoted by "-").

Plus ("+") sequences may be composed of non-specific hybridizing nucleobases such as e.g. inosine. Minus ("-") sequences may be composed of a nucleobase se-

quence with one specific sequence and the need of a specific partner will be specified by another specific sequence.

E.g. nucleobase sequence I (inosine) = "+"; nucleobase sequence T (thymine) = "-", and nucleobase sequence G (guanine) = "*".

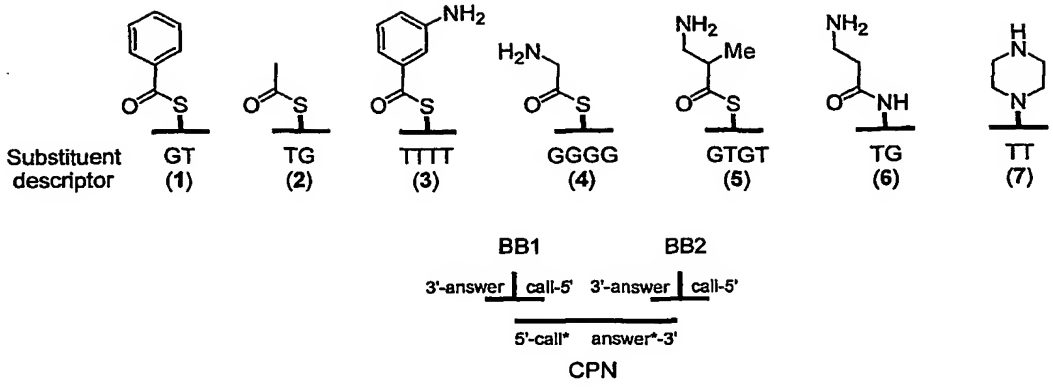
	"+": Allowed reactive group on CCPN's further downstream	"-": Disallowed reactive group on CCPN's further downstream	"*": Needed reactive group on CCPN's further downstream
CCPN sequence	I	T	G
CPN sequence accepted	A or C	A	C

As the need, acceptance or disallowance of e.g. four different reaction partners is to be signaled, the overall descriptor sequence for type and number of functional entities on a CCPN corresponds to four polynucleotide sub-regions. In the following illustrations, the regions 1, 2, 3, 4 correspond to the need or acceptance of the partners Ac (1); Al (2); C (3) and W (4). One further nucleobase in that polynucleotide sub-region may optionally indicate whether the functional entity reactive group is of donor or acceptor type. In the following nucleobase T (thymine) indicates a donor, nucleobase G (guanine) indicates an acceptor and nucleobase I (inosine) is used if donor/acceptor type is not specified.

In the design example above, the four regions 1 (Acylation), 2 (Alkylation), 3 (Cross Coupling/Suzuki) and 4 (Wittig/HWE) could be of a total of 8 nucleobases for the call region and 8 nucleobases for the answer region.

One simpler example, using a higher number of CPN's could be the following example. In this example, the call signal specifies only the need/allowed CCPN's and the answer similarly.

The CCPN's in a peptide like library composed of complementary connectors **1-7** could have the following identifier polynucleotide sequences.



The sequence of the complementary connector polynucleotides could then be:

CCPN1: 3'-GT-**GG**TITITI-5'
CCPN2: 3'-TG-**GG**TITITI-5'
CCPN3: 3'-**GT***ITTITI-TTTT*-**GG***TITITI-5'*
CCPN4: 3'-**GT**ITTITI-GGGG-**GG**TITITI-5'
CCPN5: 3'-**GT**ITTITI-GTGT-**GG**TITITI-5'
CCPN6: 3'-**GT**ITTITI-TG-5'
CCPN7: 3'-**GT**ITTITI-TT-5'

CCPN1 and CCPN2 carries only a call region and calls for acylating acceptors.
CCPN3-CCPN5 carries both an answer and a call region. The answer region specifies that it needs an acylating donor but also allows alkylating agents. The call region *specifies the call for an acylating acceptor.*
CCPN6 and CCPN7 carries only an answer region. The answer region specifies that it needs an acylating donor but also allows alkylating donors.

To generate this library, the following CPN may then fulfill the need:

CPN1: 3'-NN-CACAAC**AC**-CACACA**CC**-NN-5'

Where N denotes a variable nucleobase.

In this library all CCPN's carrying function entity groups of amino type have been specied as allowance for alkylation, but with the need for acylation.

In order to control the degree of supramolecular complex formation, terminator sequences may be added at some point in time. The concentration of which, will determine the mean distribution of how many CCPN's and CPN each complex is made of.

Such terminator sequences could in the example above be:

Terminator1: 3'-CACACACC-NN-5'
Terminator2: 3'-GTITTITI-NN-5'

Examples

The following example illustrates the use and the principle for the synthesis and identification of connector polynucleotide sequences enabling the synthesis of a small peptide.

Example 1:
Quasi-structure mediated synthesis of a small molecule that binds Integrin receptor α_V/β_{III}.

Materials:

- Purified human integrin α_V/β_{III} (Chemicon Inc.)
- Streptavidin Sepharose 6B (AmershamPharmacia)
- Nunc ImmunomoduleU8 Maxisorp (Biotecline cat# Nun-475078)
- Sheared herring DNA (Sigma)
- Bovine serum albumin (BSA)(Sigma-Aldrich)
- Taq-polymerase (Promega)
- Micro Bio-Spin 6 (Bio-Rad cat: 732-6221)
- *Fok*I, *Avr*II and *Pst*I restriction enzymes
- T7 Exonuclease

Connector polynucleotides (CPN's) and Complementary connector polynucleotides (CCPN's):

CPN1: 5'-pGCNNNNNACGCGANNNNTACGTANNNNTGTCACNNNNTCGTCANNNNNGC-3'

CPN2: 5'-pGCNNNNNTCATCTNNNNGCGTACNNNNNGC-3'

CCPN1: 5'-GCCTATGTGACGAATCTGTG-XXXXX-GATTC-Y-3'

CCPN2: 5'-Z-GAATC-XXXXX-ATGCGTACCGCGATTCATGCp-3'

CCPN3: 5'-Z-GAATC-XXXXX-CGCTGCAAGATGAATTCTGCp-3'

Linker polynucleotides for CPN amplification:

LP1: 5'-GATTCCTAGGATGCATATTACA

LP2: 3'-CTAAGGATCCTACGTATAATGTCG

LP3: 3'-GTCAATGCTGATGACGTp

LP4: 5'-CAGTTACGACTACTGCAGC

Amplification polynucleotides

AP1: 5'-B-$T_{in}T_{in}$GATT**CCTAGG**ATGCATATTACAGC-3'

AP2: 5'-CAGTTACGACTA*CTGCAG*C-3'

Underlined sequence = *Fok*I restriction site

Bold sequence = *Avr*II restriction site

Italic sequence = *Pst*I restriction site

P = 5'-phosphate

Sequencing polynucleotide:

SP: 5'-GATTCCTAGGATGCATATTAC

where X = PEG-linker, Glen research cat# 10-1918-90; B = biotin, Applied Biosystems and Y = 3'-amino-group, Glen research cat#20-2958-01, Z = amino modifier, Glen research cat#10-1905-90 suitable for attachment of chemical entities. p = 5'-phosphate.

Protocol

In the following protocol, the guanidine functionality of arginine may be appropriately protected if needed. E.g. by use of trifluoroacetyl (which can be removed, when needed, by alkaline treatment), benzyloxycarbonyl (which can be removed, when needed, by catalytic hydrogenation), enzymatically cleavable protecting groups and others known to the person skilled in the art.

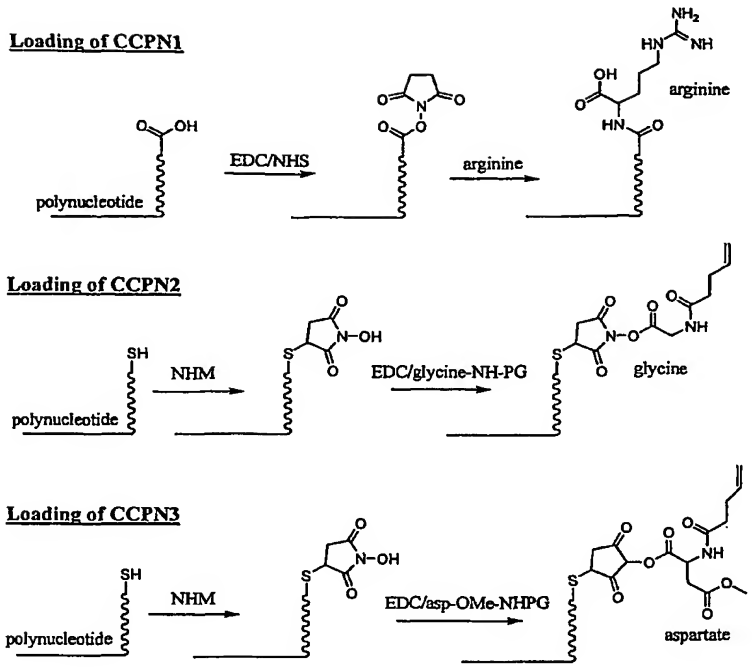
Step1: Loading of building block polynucleotides

CCPN1.

5 nmol of CCPN1 is incubated with 25 mM NHS and 50 mM EDC in 100 mM HEPES-OH buffer pH 7.5 at 30 °C for 30 min. Excess EDC/NHS is removed using spin-column filtration. The NHS-activated CCPN1 is incubated with 20 mM arginine in HEPES-OH buffer at 30 °C for 2 hours. CCPN1 is purified using spin column filtration and loading efficiency is tested using ES-MS (Bruker Inc.)

CCPN2 & CCPN3.

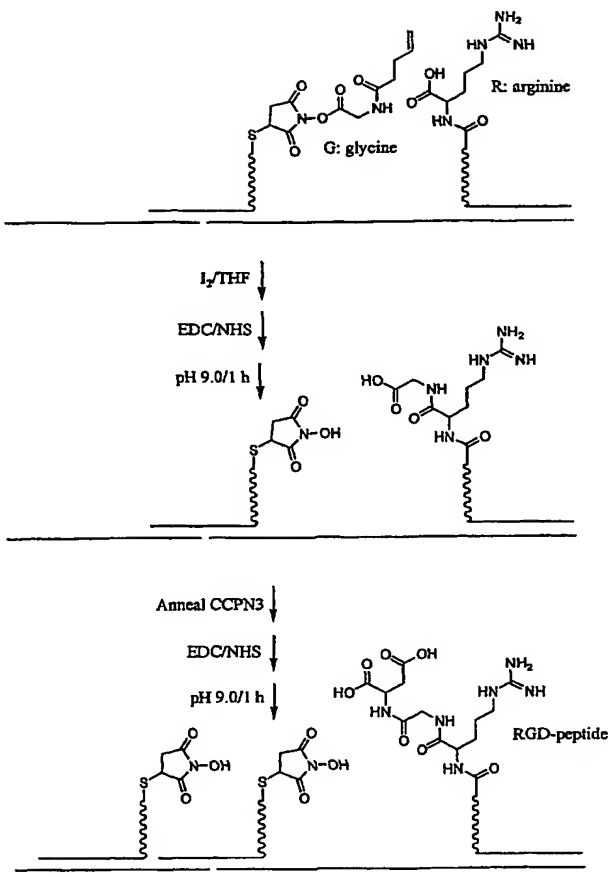
5 nmol of CCPN2 or CCPN3 is incubated with 25 mM TCEP [tricarboxyethylphosphine] in 100 mM HEPES-OH at 30 °C for 1 hour producing a terminal SH-group. TCEP and buffer are removed by gel-filtration before addition of 50 mM N-hydroxymaleimide (NHM) in 100 mM HEPES-OH, pH 7.5. The preparations are incubated at 30 °C for 2 hours producing CCPN's comprising a NHS activating unit. Excess NHM is removed by gel-filtration. 100 mM 4-pentenoyl glycine or 4-pentenoyl-OMe aspartate in DMF is pre-activated using equimolar EDC in DMF at 25 °C for 30 minutes. The CCPN-NHS is incubated with 50 mM EDC activated 4-pentenoyl protected glycine or 4-pentenoyl-OMe aspartate, respectively, in a 100 mM MES buffer pH 6.0 at 25 °C for 5 minutes (DMF:H_2O = 1:4). Excess building block is removed by gel-filtration and activated CCPN is eluted in 100 mM MES buffer pH 6.0.



Scheme 1: Loading of polynucleotides with building blocks

Step 2: Formation of multi-polynucleotide complexes and transfer of building blocks.

10 pmol each of activated CCPN1 and CCPN2 from step 1 is incubated with 10 pmol of CPN1 and CPN2 in 100 mM MES buffer pH 6.0 supplemented with 5 mM I_2 in THF (for amino-deprotection). The reaction is incubated at 25 °C for 4 hours allowing assembly of multi-polynucleotide complexes and concomitant transfer of the glycine residue (Scheme 2B). Subsequently, 10 pmol of activated CCPN3 is added to the reaction and incubated at 25 °C for an additional 4 hours. Transfer of the O-Methyl aspartate followed by mild alkaline treatment (pH 9.0, 1h) produce the RGD peptide linked to CCPN1 (Scheme 2C).



Scheme 2: Quasi-structure mediated synthesis of an RGD peptide

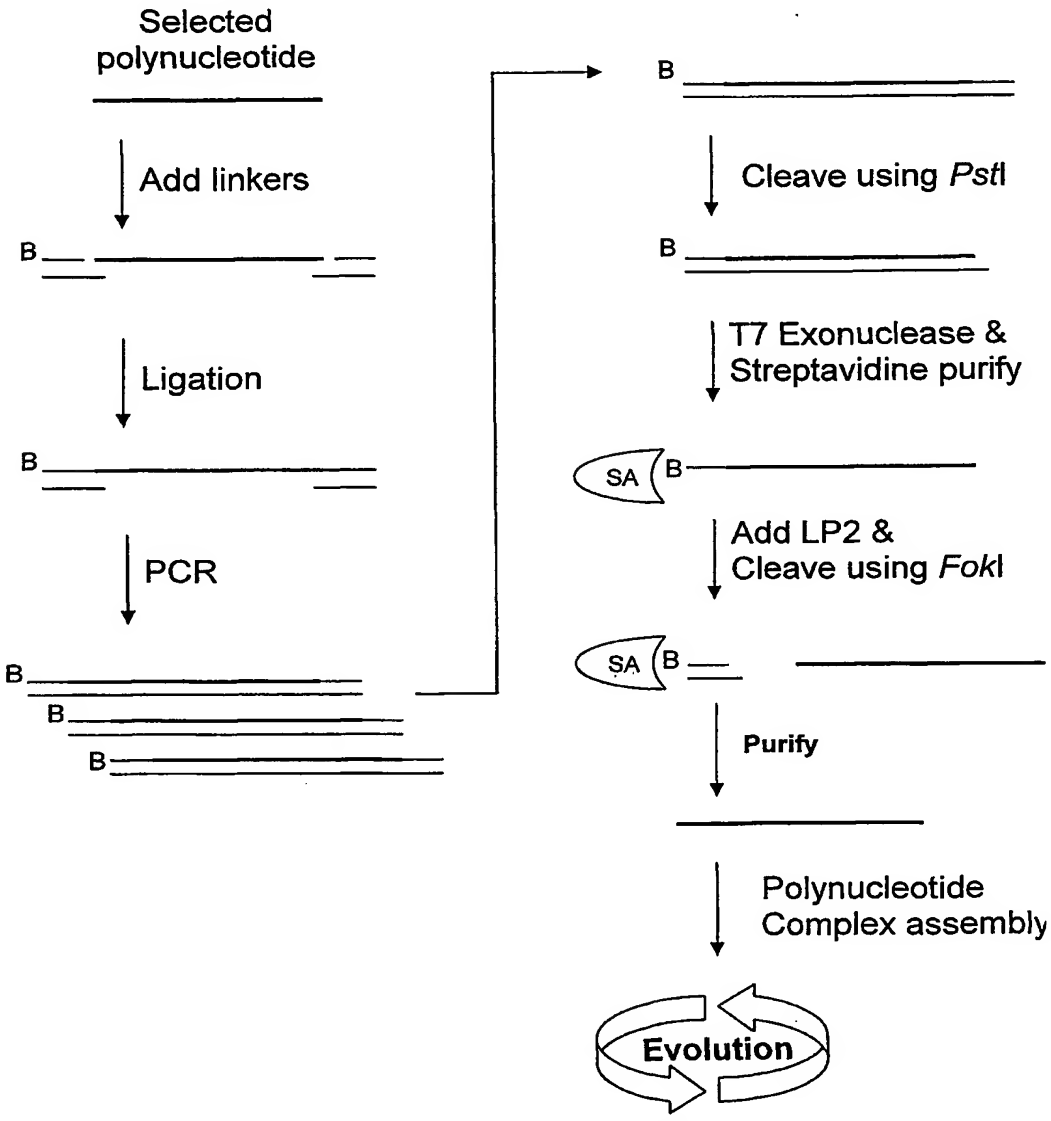
Step 3: Selection of multi-polynucleotide complexes displaying the RGD peptide.

A single well of a Nunc-8 plate is incubated overnight with 100 µl of 1 µg/ml of integrin receptor in standard phosphate-buffered saline (PBS). The well is washed five times with 100 µl PBS. The well is blocked using 100 µl 0.5mg/ml sheared herring DNA in PBS-buffer for 2 h at room temperature.
Finally the well is washed five times using 100 µl Integrin binding buffer [Tris-HCl (pH 7.5), 137 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 1 mM $MnCl_2$].
The multi-polynucleotide complexes are added to the immobilised integrin and incubated at 37 °C for 30 min. The supernatant is removed and the immobilised integrin is washed 5 times using 100 µl Integrin binding buffer. The polynucleotide com-

plexes are eluted heating the sample to 80 °C for 5 min. The sample is cooled to room-temperature.

Step 4: Amplification of polynucleotides

1 µl of the sample from step 3 is used for amplification of polynucleotide fragments using the following protocol (see also Scheme 3):



Scheme 3: Amplification of connector polynucleotides

1 pmol each of preformed LP1/LP2 complex and 1 pmol of LP3/LP4 complex is added to the eluted connector polynucleotide fragments in ligation buffer comprising 30 mM Tris-HCl (pH 7.8) 10 mM $MgCl_2$, 10mM DTT and 1 mM dATP before addition of 10 units of T4 DNA ligase. The sample is incubated at 16°C for 4 hours before denaturation at 75 °C for 15 min. 1/10 of the sample is used as template in a PCR reaction comprising 10 pmol of the oligonucleotides AP1 and AP2
10 mM Tris-HCl pH 9.0, 50 mM KCl, 1 mM $MgCl_2$, 0.1 % Triton X-100, 250 mM each of dATP, dCTP, dGTP and dTTP. The sample is run with initial denaturation at 94 °C, for 2 min and 30 cycles using denaturation at 94°C for 30 seconds, annealing at 44 °C for 30 seconds and elongation at 72°C for 15 seconds. Finally, the sample is phenol extracted twice before DNA precipitation.
Regeneration of singlestranded connector polynucleotides are accomplished by first cleaving the PCR products using 10 units of *PstI* in a buffer comprising 50 mM Tris-HCl (pH 7.9),100 mM NaCl, 10 mM $MgCl_2$ and 1 mM DTT at 37 °C for 2 hours in a volume of 50 µl. Following cleavage, the sample is subjected to 5' to 3' digestion using T7 exonuclease at 37 °C for 1 hour in a total volume of 500 µl. Next, the biotinylated strand is purified on streptavidine-sepharose beads using the following procedure:
50 streptavidine-sepharose slurry is washed 4 times using 1 ml of 20 mM NH_4-acetate pH 7.5 before addition of digestion sample in a total volume of 500 µl and further incubation at 25 °C for 15 minutes. The streptavidine beads are washed 4 times using 1 ml of H_2O. The amplified polynucleotides are regenerated by annealing of 10 pmol of LP2 to the streptavidine bound polynucleotide. Excess LP2 is removed by washing the beads 4 times using H_2O. Subsequently, the beads are incubated in 100 µl buffer comprising 20 mM Tris-acetate (pH 7.9), 50 mM K-acetate, 10 mM $MgCl_2$ and 1 mM DTT before addition of 10 units of *FokI* restriction enzyme and incubation at 37 °C for 2 hours. The eluted polynucleotide is sampled and heated for 80 °C for 5 minutes to denature the restriction enzyme before purification of the polynucleotides using gel-filtration.

Step 5: Repeat step 2 using the amplified polynucleotides

The new population of single stranded polynucleotides which are enriched for sequences that represent ligands for the integrin αV/β3 receptor are annealed to the library of tagged-peptides from step1 as described in step 2 and subjected to yet another round of selection and amplification.

The selection and amplification procedure (step2-5) is repeated for 5 rounds.

Step 6: Identification of connector polynucleotide sequences involved in the synthesis of RGD.

The identity of enriched double stranded polynucleotide fragments from step 4 is established by DNA cloning in a M13mp18 plasmid vector and examining individual clones by sequence analysis.

For statistical purposes more than 50 clones is sequenced to identify sequence bias within the pool of cloned polynucleotides.

Example 2:

In the following, a zipper box designates a polynucleotide based region within the linker of the CCPN, which may hybridize to complementary polynucleotide based regions of other CCPN's. Alternatively, this zipperbox may hybridize to a CPN. Such hybridizations will allow the functional entities of two individual CCPN's to reach high proximity (Figure **21**).

In the following examples, CCPN building blocks are used which contain a zipper box adjacent to the functional entity. The zipper box sequences are underlined below. The following buffers and protocols are used in the same examples.

Buffers.

Buffer A (100 mM Hepes pH= 7,5; 1 M NaCl)

5'-Labeling with ^{32}P.

Mix 5 pmol oligonucleotide, 2 µl 10 x phosphorylation buffer (Promega cat#4103), 1 µl T4 Polynucleotide Kinase (Promega cat#4103), 1 µl γ-^{32}P ATP, add H_2O to 20 µl. Incubate at 37°C 10-30 minutes.

PAGE (polyacrylamide gel electrophoresis).

The samples are mixed with formamide dye 1:1 (98% formamide, 10 mM EDTA, pH 8, 0,025 % Xylene Cyanol, 0,025% Bromphenol Blue), incubated at 80°C for 2 minutes, and run on a denaturing 10% polyacrylamide gel. Develop gel using autoradiography (Kodak, BioMax film).

DNA-oligos:

Zipper box sequences are underlined. Note that when the CCPN building block zipper boxes interact with zipper boxes in the CPN, the length of the zipper box duplex is one nucleotide longer than is underlined.

X= Carboxy-dT Glenn Research cat.no. 10-1035-
Z = Amino-Modifier C6 dT Glenn Research cat.no. 10-1039-
6= Amino-Modifier 5 Glenn Research cat. no. 10-1905
9= Spacer 9 Glenn Research cat. no. 10-1909
P= PC-spacer
B= Biotin

AH140: 5'-
AGCTGGATGCTCGACAGGTCAGGTCGATCCGCGTTACCAGTCTTGCCTGAACG
TAGTCGTCCGATGCAATCCAGAGGTCG
AH 154: 5'-
AGCTGGATGCTCGACAGGTCAAGTAACAGGTCGATCCGCGTTACCAGTCTTGC
CTGAACGTAGTCGTCCGATGCAATCCAGAGGTCG
AH 155: 5'-
CTGGTAACGCGGATCGACCTGTTACX
AH 202: 5'-TCTGGATTGCATCGGGTTACX
AH 251: 5'- ZGACCTGTCGAGCATCCAGCTPB
AH 270: 5'- 6GTAACGACCTGTCGAGCATCCAGCT
AH 272: 5'-ACGACTACGTTCAGGCAAGAGTTACX
AH 284: 5'-
AGCTGGATGCTCGACAGGTCAAGTAACAGGTCGATCCGCGTTATATCGTTTAC
GGCATTACCGCCCATAGCTTGCGGCTTGC

AH 292: 5'-
GGCATGGTCCATCGACTGCAATATGCAAGCCGCAAGCTATGGGC
AH 293: 5'-
GGCATGGTCCATCGACTGCAATATCGTATAGCAAGCCGCAAGCTATGGGC
AH 294: 5'-
GGCATGGTCCATCGACTGCAATATCGTTTACGGCATTACCGCAAGCCGCAAG-
CTATGGGC
AH 295: 5'-
GGCATGGTCCATCGACTGCAATATCGTTTACGGCATTACCATATCGTT-
TACGGCATTACCGCAAGCCGCAAGCTATGGGC
AH 296: 5'-
GGCATGGTCCATCGACTGCAATATCGTTTACGGCATTACCATATCGTTTACGGC
ATTACCATATCGTTTACGGCATTACCGCAAGCCGCAAGCTATGGGC
AH 298: 5'-
GGCATGGTCCATCGACTGCAGCAAGCCGCAAGCTATGGGC
AH 325: 5'-CTTATACCTTGTTGTAGCCG
TCTTGCCTGAACGTAGTCGTCCGATGCAATCCAGAGGTCG
AH 326: 5'-CTTATACCTTGTTGTAGCCG
TCTTGCCTGAACGTAGTCGTTTCCGATGCAATCCAGAGGTCG
AH 327: 5'-CTTATACCTTGTTGTAGCCG
TCTTGCCTGAACGTAGTCGTACTTCCGATGCAATCCAGAGGTCG
AH 328: 5'-CTTATACCTTGTTGTAGCCG
TCTTGCCTGAACGTAGTCGTTGACTTCCGATGCAATCCAGAGGTCG
AH 329: 5'-
CTTATACCTTGTTGTAGCCGTCTTGCCTGAACGTAGTCGTGGTGACTTC-
CGATGCAATCCAGAGGTCG
AH330: 5'-
CGGCTACAACAAGGTATAAGAAAAACATCGTAGGATTCTTTCCTACGATGG-
CAAGCCGCAAGCTATGGGC
AH332: 5'-
CGGCTACAACAAGGTATAAGAAAAACAGGATTCTTTCCTGGCAAGCCGCAAG-
CTATGGGC

AH 351: 5'-
CTTATACCTTGTTGTAGCCGTCTTGCCTGAACGTAGTCGTGGTGACTTGGC-
CGATGCAATCCAGAGGTCG
AH 352: 5'-
CTTATACCTTGTTGTAGCCGTCTTGCCTGAACGTAGTCGTGGTGACTTGGT-
GCCGATGCAATCCAGAGGTCG
AH 353: 5'-
CTTATACCTTGTTGTAGCCGTCTTGCCTGAACGTAGTCGTGGTGACTTGGT-
GACCCGATGCAATCCAGAGGTCG
AH 354: 5'-
CTTATACCTTGTTGTAGCCGTCTTGCCTGAACGTAGTCGTGGTGACTTGGT-
GACTTCCGATGCAATCCAGAGGTCG
AH 355: 5'-
CTTATACCTTGTTGTAGCCGTCTTGCCTGAACGTAGTCGTGGTGACTTGGT-
GACTTGGCCGATGCAATCCAGAGGTCG
AH 378: 5'-TGCAGTCGATGGACCATGCCAGCTGGATGCTCGACAGGTC AAC-
CGATGCAATCCAGAGGTCG
AH 379: 5'-TGCAGTCGATGGACCATGCCAGCTGGATGCTCGACAGGTC AAT-
CAGGCTGCCGATGCAATCCAGAGGTCG
AH 380: 5'-CGGTTGAGGTACAGGTCGATCCGCGTTACCAG TCTTGCCTGAACG-
TAGTCGTGCCCATAGCTTGCGGCTTGC
AH 381: 5'- 69GTAACGTACCTCAACCGGACCTGTCGAGCATCCAGCT
AH 382: 5'-GGTACAGGTCGATCCGCGTTACCAG TCTTGCCTGAACG-
TAGTCGTGCCCATAGCTTGCGGCTTGC
AH 383: 5'-GGTACAGGTCGATCCGCGTTACCAG GGTACTCTTGCCTGAACG-
TAGTCGTGCCCATAGCTTGCGGCTTGC
AH 386: 5'-
GTTGAGGTACAGGTCGATCCGCGTTACCAGTCTTGCCTGAACGTAGTCGT-
GCCCATAGCTTGCGGCTTGC
AH 387: 5'-
TGAGGTACAGGTCGATCCGCGTTACCAGTCTTGCCTGAACGTAGTCGTGC-
CCATAGCTTGCGGCTTGC
AH 388: 5'-
AGGTACAGGTCGATCCGCGTTACCAGTCTTGCCTGAACGTAGTCGTGCC-
CATAGCTTGCGGCTTGC

AH 392: 5'-CGACCTCTGGATTGCATCGGGTTACZ
AH 393: 5'-ACGACTACGTTCAGGCAAGAGTTACZ
AH 394: 5'-CTGGTAACGCGGATCGACCTGTTACZ

The oligonucleotides were prepared by conventional phosphoramidite synthesis.

Example 2A:

We wanted to examine whether the cross-linking efficiency could be increased by using CPN/CCPN-sequences that allow the formation of higher order structures (see figure 27). First, we designed two of the CCPNs (the T2 sequences AH330 and AH332) as hair-pin structures, in the hope that this structure would increase the proximity of the CCPNs that must react (here AH251 and AH202). To further test the structural requirements, we also tested different spacings of the T1 oligos (spacings of 20, 22, 24, 26 and 28 nt were examined in this example). The spacing referred to is the distance between the region of T1 that anneals to AH202 and the region of T1 that anneals to T2 (see figure 27).
This experiment also is an example of the oligonucleotide complex depicted in "Figure 4, claim 1".

Experimental. Mix 10 µl Buffer A, relevant oligos in various concentrations (1 pmol oligo 1, 10 pmol oligo 2, 3 pmol oligo 3, 5 pmol oligo 4 and 8 pmol oligo 5 (See table I, below), and add H_2O to 50 µl.

Table I:

Experiment	Oligo 1 (^{P}-32-labelled) BB1	Oligo 2 BB0	Oligo 3 CPN T1	Oligo 4 CCPN T2	Oligo 5 CPN T3
1	AH 202	AH 251		AH 154	
2	AH 202	AH 251	AH 325 (20 nt)	AH 330 (10 nt)	AH 284 (20 nt)
3	AH 202	AH 251	AH 326 (22 nt)	AH 330 (10 nt)	AH 284 (20 nt)
4	AH 202	AH 251	AH 327 (24 nt)	AH 330 (10 nt)	AH 284 (20 nt)
5	AH 202	AH 251	AH 328 (26 nt)	AH 330 (10 nt)	AH 284 (20 nt)
6	AH 202	AH 251	AH 329 (28 nt)	AH 330 (10 nt)	AH 284 (20 nt)
7	AH 202	AH 251	AH 325 (20 nt)	AH 332 (5 nt)	AH 284 (20 nt)
8	AH 202	AH 251	AH 326 (22 nt)	AH 332 (5 nt)	AH 284 (20 nt)
9	AH 202	AH 251	AH 327 (24 nt)	AH 332 (5 nt)	AH 284 (20 nt)
10	AH 202	AH 251	AH 328 (26 nt)	AH 332 (5 nt)	AH 284 (20 nt)
11	AH 202	AH 251	AH 329 (28 nt)	AH 332 (5 nt)	AH 284 (20 nt)

Anneal from 80°C to 30°C (-1°C/ min). Add 0,5 M DMT-MM. (Prepared according to Kunishima et al. Tetrahedron (2001), 57, 1551) dissolved in H_2O, to a final concentration of 50 mM. Incubate at 30°C o/n. Analyze by 10% urea polyacrylamide gel electrophoresis.

The expected complexes formed are shown in figure 27; results are shown in figure 28.

Results. As can be seen in figure 28, very efficient cross-link (reaction of amino group of oligo AH251 with carboxylic acid of oligo AH202) is obtained for certain combinations of T1, T2, and T3:

- A control reaction (AH202 and AH251 annealed to AH154) shows 20-40% efficient cross-link (figure 28, lane 1).
- Using the T2 oligo AH330, with a 10 bp duplex in the hair-pin structure, efficient cross-link between AH251 and AH202 is observed for only the CPN T1 with 28 nt spacing (AH329) (figure 28, lane 6). The cross-linking efficiency is almost as high as observed in the simple control reaction (compare lanes 1 and 6). None of the spacings 20, 22, 24, 26 nt (lanes 2-5) lead to efficient cross-links.
- The same pattern is observed when using the T2 oligo AH332 with a 5 bp duplex in the hair-pin structure, i.e. only the T1 oligo with a 28 nt spacing (AH329) provides efficient crosslinking. The cross-linking efficiency is almost as high as observed in the simple control reaction (compare lanes 1 and 11).

Thus, from the experiments of figure 28 it is concluded that efficient encoded reactions may be obtained by appropriate design of CPN and CCPN.

Example 2B:

Example 2A shows that by incorporating sequences that allow T2 to form a hair-pin structure, the reaction efficiencies may be rather high. We wanted to examine this further. Thus, we next tested additional spacings of the T1 sequence.

Experimental. Mix 2 µl Buffer A, relevant oligos in various concentrations (0,2 pmol oligo 1, 2 pmol oligo 2, 0,6 pmol oligo 3, 1 pmol oligo 4 and 1,6 pmol oligo 5 (See table II, below), and add H_2O to 10 µl.

Table II:

Experiment	Oligo 1 (^{P}-32-labelled) BB1	Oligo 2 BB0	Oligo 3 CPN T1	Oligo 4 CCPN T2	Oligo 5 CPN T3
1	AH 202	AH 251	AH 328 (26 nt)	AH 330 (10 nt)	AH 284 (20 nt)
2	AH 202	AH 251	AH 329 (28 nt)	AH 330 (10 nt)	AH 284 (20 nt)
3	AH 202	AH 251	AH 351 (30 nt)	AH 330 (10 nt)	AH 284 (20 nt)
4	AH 202	AH 251	AH 352 (32 nt)	AH 330 (10 nt)	AH 284 (20 nt)
5	AH 202	AH 251	AH 353 (34 nt)	AH 330 (10 nt)	AH 284 (20 nt)
6	AH 202	AH 251	AH 354 (36 nt)	AH 330 (10 nt)	AH 284 (20 nt)
7	AH 202	AH 251	AH 355 (38 nt)	AH 330 (10 nt)	AH 284 (20 nt)
8	AH 202	AH 251		AH 154	

Anneal from 80°C to 30°C (-1°C/ min). Add 0,5 M DMT-MM. (Prepared according to Kunishima et al. Tetrahedron (2001), 57, 1551) dissolved in H_2O, to a final concentration of 50 mM. Incubate at 30°C o/n.
Analyze by 10% urea polyacrylamide gel electrophoresis.

The results are shown in figure 29. The conclusions are:

- The control reaction (AH202 and AH251 annealed to AH154) shows 20-40% efficient cross-link (figure 29, lane 8).
- Spacings of 28, 30, 32 and 38 nt give efficient cross-linking (figure 29, lanes 2, 3, 4 and 7); spacings of 26, 34 and 36 nt give poor efficiencies.
- The spacing of 28 nt provide the highest efficiency.

It is thus concluded that a CPN T1 with 28 nt spacing provides the highest cross-linking of the spacings tested.

Examples 2C-2F:

We wanted to test a set-up including 5 CCPNs and 2 CPNs (see figure 31). This set-up includes a CCPN (AH381) with a linker sequence that is complementary to the 5'-terminal region of CPN T3. We hypothesize that this leads to formation of the higher order structure shown in the lower half of figure 31 by annealing of the linker of CCPN0 (AH381) with the 5'terminus of CPN T3.

The CPNs and CCPNs used in this experiment have the following features:

CPN T1: Contains annealing regions for CCPN1, CCPN0 and CCPN T2. The spacing between the annealing region for CCPN1 and CCPN0 is either 2 or 10 nt.

CPN T3: Contains annealing regions for CCPN2, CCPN3 and CCPN T2. In addition, the 5'end contains a region complementary to the linker of CCPN0. The regions of complementarity consist of 5, 6, 8, 10, or 12 nt for AH382, AH388, AH387, AH386 and AH380, respectively. AH383 contains at its 5'-end a complementarity region of 5 nt, as well as a region of 5 nucleotides (between the regions annealing to CCPN2 and CCPN3) that is also complementary to the linker of CCPN0.

Figure 30 shows how this set-up may be used to encode the synthesis of a small molecule with 4 encoded functional entities. Thus, in a step-wise fashion, the reaction of CCPN0 and CCPN1 is first conducted in the presence of CPN T1, in the absence of CCPN T2 and CPN T3. Then CCPN T2, CPN T3 and CCPN2 is added, and the reaction between CCPN0 and CCPN2 is performed. Finally, CCPN3 is added, and the reaction between CCPN0 and CCPN3 is performed.

Example 2C:

We first tested step 1, i.e. the reaction between CCPN0 and CCPN1 in the presence of CPN T1, by performing a cross-link reaction between the amino group of CCPN0 and the carboxy group of CCPN1 (see figure 30 and 31).

Experimental. Mix 2 µl Buffer A, relevant oligos in various concentrations (0,2 pmol oligo 1, 2 pmol oligo 2, 1 pmol oligo 3 (See table III), and add H_2O to 50 µl.

Table III:

Experiment	Oligo 1 (^{P}-32-labelled) CCPN 1	Oligo 2 CCPN 0	Oligo 3 CPN T1
1	AH 202	AH 381	AH 379
2	AH 202	AH 381	-
3	AH 202	AH 270	AH 140
4	AH 202	AH 270	-

Anneal from 80°C to 30°C (-1°C/ min.). Dilute 100 times and then add 0,5 M DMT-MM (Prepared according to Kunishima et al. Tetrahedron (2001), 57, 1551) dissolved in H_2O, to a final concentration of 50 mM. Incubate at 10°C for 5 sec, and 35° C for 1 sec. Repeat o/n.
Analyze by 10% urea polyacrylamide gel electrophoresis.

Results. As can be seen in figure 32, the reaction efficiency is high (approximately 50-60%).

Example 2D:

We next tested steps 2 and 3 (see figure 30 and 31), i.e. the reaction between CCPN0 and CCPN2, and the reaction between CCPN0 and CCPN3, respectively.

Experimental. Mix 10 µl Buffer A, relevant oligos in various concentrations (1 pmol oligo 1, 10 pmol oligo 2, 8 pmol oligo 3, 6 pmol oligo 4 and 4 pmol oligo 5 (See table IV, below), and add H_2O to 50 µl.

Table IV:

Experiment	Oligo 1 (^{P}-32-labelled) CCPN 2 or CCPN 3	Oligo 2 CCPN 0	Oligo 3 CPN T1	Oligo 4 CCPN T2	Oligo 5 CPN T3
1	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 380 (12 nt)
2	AH 155	AH 381	AH 378	-	AH 380 (12 nt)
3	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 382 (5 nt)
4	AH 272	AH 381	AH 378	AH 294 (20 nt)	AH 382 (5 nt)
5	AH 155	AH 381	AH 378	-	AH 382 (5 nt)
6	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 383 (5nt+5 nt)
7	AH 272	AH 381	AH 378	AH 294 (20 nt)	AH 383 (5nt+5 nt)
8	AH 155	AH 381	AH 378	AH 292 (4 nt)	AH 382 (5 nt)
9	AH 155	AH 381	AH 378	AH 296 (60 nt)	AH 382 (5 nt)
10	AH 155	AH 381	AH 140		
11	AH 272	AH 381	AH 140		

Anneal from 80°C to 30°C (-1°C/ 30 sec.). Dilute 100 times and then add 0,5 M DMT-MM. (Prepared according to Kunishima et al. Tetrahedron (2001), 57, 1551) dissolved in H_2O, to a final concentration of 50 mM. Incubate at 10°C for 5 sec, and 35° C for 1 sec. Repeat o/n.

Analyze by 10% urea polyacrylamide gel electrophoresis.

Results. From figure 33, it may be concluded that

- Using CPN T3 with a 5 nt complementarity region at its 5'end, no significant cross-linking is observed for any oligo combination tested (figure 33, lanes 3-9).
- Using CPN T3 with a 12 nt complementarity region, an efficient cross-linking between CCPN0 and CCPN3 is observed (figure 33, lane 1)(CCPN2 was not tested in this experiment). When the CCPN T2 is excluded, much less cross-linking is observed, indicating that the reaction is dependent on the presence of CCPN T2.
- Lanes 10 and 11 show the control reactions.
- The same experiments were performed under constant reaction temperatures of either 15 °C or 25 °C (rather than alternating between 10 and 35 °C). Similar results were obtained, except that more efficient reactions were obtained in the absence of CCPN T2 (data not shown).

Example 2E:

As a continuation of the experiments in example 4, a number of parameters (spacing between annealing regions, length of complementarity regions, and dependency of CCPN T2) were now examined as regards the effect on cross-linking efficiency of step 2 and 3.

Experimental. Mix 10 µl Buffer A, relevant oligos in various concentrations (1 pmol oligo 1, 10 pmol oligo 2, 8 pmol oligo 3, 6 pmol oligo 4 and 4 pmol oligo 5 (See table V, below), and add H_2O to 50 µl.

Table V:

Experiment	Oligo 1 (^{P}-32-labelled) CCPN 2 or CCPN 3	Oligo 2 CCPN 0	Oligo 3 CPN T1	Oligo 4 CCPN T2	Oligo 5 CPN T3
1	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 380 (12 nt)
2	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 380 (12 nt)
3	AH 155	AH 381	AH 379	AH 294 (20 nt)	AH 380 (12 nt)
4	AH 155	AH 381	AH 379	AH 294 (20 nt)	AH 380 (12 nt)
5	AH 272	AH 381	AH 379	AH 294 (20 nt)	AH 380 (12 nt)
6	AH 272	AH 381	AH 379	AH 294 (20 nt)	AH 380 (12 nt)
7	AH 155	AH 381	AH 379		AH 380 (12 nt)
8	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 386 (10 nt)
9	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 387 (8 nt)
10	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 388 (6 nt)
11	AH 272	AH 381	AH 378	AH 294 (20 nt)	AH 387 (8 nt)
12	AH 272	AH 381	AH 378	AH 294 (20 nt)	AH 387 (8 nt)
13	AH 272	AH 381	AH 379	AH 294 (20 nt)	AH 387 (8 nt)
14	AH 272	AH 381	AH 379	AH 294 (20 nt)	AH 387 (8 nt)

15	AH 272	AH 381	AH 378	AH 294 (20 nt)	AH 388 (6 nt)
16	AH 272	AH 381	AH 378	AH 294 (20 nt)	AH 388 (6 nt)
17	AH 155	AH 381	AH 140	-	-
18	AH 272	AH 381	AH 140	-	-
19	AH 155	AH 381	-	-	-
20	AH 155	AH 381	AH 378	-	AH 386 (10 nt)
21	AH 155	AH 381	AH 378	AH 298 (0 nt)	AH 386 (10 nt)
22	AH 155	AH 381	AH 378	AH 292 (4 nt)	AH 386 (10 nt)
23	AH 155	AH 381	AH 378	AH 293 (10 nt)	AH 386 (10 nt)
24	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 386 (10 nt)
25	AH 155	AH 381	AH 378	AH 295 (40 nt)	AH 386 (10 nt)
26	AH 155	AH 381	AH 378	AH 296 (60 nt)	AH 386 (10 nt)
27	AH 155	AH 381	AH 378	AH 294 (20 nt)	AH 382 (5 nt)

Anneal from 80°C to 20°C (-1°C/ min.). Dilute 100 times and then add 0,5 M DMT-MM. (Prepared according to Kunishima et al. Tetrahedron (2001), 57, 1551) dissolved in H_2O, to a final concentration of 50 mM. Incubate at 10°C for 5 sec, and 35° C for 1 sec. Repeat o/n.

Analyze by 10% urea polyacrylamide gel electrophoresis.

Results (figure 34).

- A 5'-complementarity region of CPN T3 of 12 nt provides efficient cross-linking, whereas 10, 8, 6 or 5 nt complementarity regions provide little or no cross-linking efficiency (figure 34, compare lanes 1, 8, 9, 10, and 27).

- The cross-linking reaction is strongly dependent on the presence of CCPN T2 (figure 34, compare lanes 3 and 7).
- The presence of CCPN1, annealed to CPN T1, does not decrease the cross-linking efficiency of CCPN0 with either of CCPN2 or CCPN3 (figure 34, compare lanes 1 and 2, lanes 3 and 4, lanes 5 and 6).
- The reaction of CCPN0 with CCPN2 and with CCPN3 is approximately of same efficiency (figure 34, compare lanes 4 and 6, lanes 3 and 5).
- Spacings of either 2 nt or 10 nt in CPN T1 both provide efficient cross-linking (figure 34, lanes 1-4).
- A spacing of more than 20 nt in CCPN T2 is required for obtaining efficient cross-linking (figure 34, lanes 24-26). Spacings of 0, 4, or 10 nt provide no cross-reaction (figure 34, lanes 21-23).

Example 2F:

In the examples above it is concluded that the complementarity region of CPN T3 must be at least 12 nt in order to obtain efficient cross-linking. We wanted to examine whether shorter complementarity regions (in CPN T3) would be efficient if combined with longer spacing regions (in CCPN T2).

Experimental. Mix 2 µl Buffer A, relevant oligos in various concentrations (0,2 pmol oligo 1, 1 pmol oligo 2, 0,8 pmol oligo 3, 0,6 pmol oligo 4 and 0,4 pmol oligo 5 (See table VI, below), and add H_2O to 50 µl.

Table VI:

Experiment	Oligo 1 (^{P}-32-labelled) CCPN 2 or CCPN 3	Oligo 2 CCPN 0	Oligo 2b CCPN 1	Oligo 3 CPN T1	Oligo 4 CCPN T2	Oligo 5 CPN T3
1	AH 155	AH 381		AH 140		
2	AH 155	AH 381	AH 202	AH 379	AH 294 (20 nt)	AH 380 (12 nt)
3	AH 155	AH 381	AH 202	AH 379	AH 294 (20 nt)	AH 387 (8 nt)
4	AH 155	AH 381	AH 202	AH 379	AH 294 (20 nt)	AH 382 (5 nt)
5	AH 155	AH 381	AH 202	AH 379	AH 296 (60 nt)	AH 380 (12 nt)
6	AH 155	AH 381	AH 202	AH 379	AH 296 (60 nt)	AH 387 (8 nt)
7	AH 155	AH 381	AH 202	AH 379	AH 296 (60 nt)	AH 382 (5 nt)
8	AH 155	AH 381	AH 202	AH 379	AH 295 (40 nt)	AH 380 (12 nt)
9	AH 155	AH 381	AH 202	AH 379	AH 295 (40 nt)	AH 387 (8 nt)
10	AH 155	AH 381	AH 202	AH 379	AH 295 (40 nt)	AH 382 (5 nt)
11	AH 155	AH 381	AH 202	AH 379		AH 380 (12 nt)
12	AH 155	AH 381	AH 202	AH 379		AH 387 (8 nt)
13	AH 155	AH 381	AH 202	AH 379		AH 382 (5 nt)

Anneal from 80°C to 20°C (-1°C/ min.). Dilute 100 times and then add 0,5 M DMT-MM (Prepared according to Kunishima et al. Tetrahedron (2001), 57, 1551) dis-

solved in H_2O, to a final concentration of 50 mM. Incubate at 10°C for 5 sec, and 35° C for 1 sec. Repeat o/n.
Analyze by 10% urea polyacrylamide gel electrophoresis.

Results (figure 35).

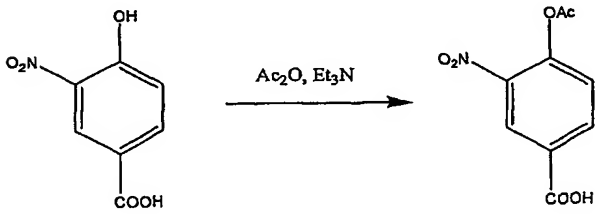
- A complementarity region (5'-end of CPN T3) of 12 nt (rather than 5 or 8 nt) provides a more efficient reaction for all CCPN T2 spacings tested (figure 9, compare lanes 2, 3 and 4; lanes 5, 6, and 7; lanes 8, 9 and 10)

Example 2G: Synthesis of a small molecule through the reaction of functional entity reactive groups on three CCPN's.

In this example the set-up described in figure 30 is employed to synthesize a small molecule, where three chemical moieties are combined by the CPNs and CCPNs. This is also an example of the oligonucleotide complex depicted in "figure 4, claim 2" (see also figure 36 for explanation). Finally, this is also an example of circular structures such as depicted in "Figure 4, claim 1, 7-8, and 10-11.

Experimental.

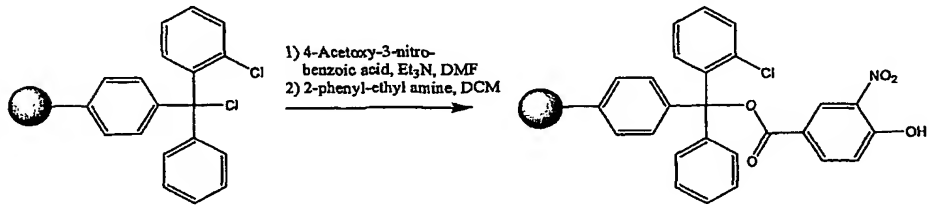
Synthesis of functional entities.



4-Acetoxy-3-nitro-benzoic acid

4-Hydroxy-3-nitro-benzoic acid (5.49 g, 30 mmol) was dissolved in acetone (10 ml), triethylamine (10 ml) and acetic acid anhydride (5.67 ml, 60 mmol). The solution was stirred for 24h at rt. The reaction mixture was added dichloromethane (100 ml), ice

(20 g) and acidified by addition of concentrated hydrochloric acid. The aqueous phase was extracted with dichloromethane (2 x 25 ml). The combined organic phases were stirred with sodium sulphate added activated carbon, filtered and evaporated. Recrystallisation from EtoAc:Heptane gave 3.45 g (51%) pure material.
NMR ($CDCl_3$): δ 8.84 (d, 1H), 8.40 (dd, 1H), 7.41 (d, 1H) and 2.44 (s, 3H).



4-hydroxy-3-nitro-benzoic acid-(2-chloro-tritylresin) ester

2-chlorotrityl chloride resin (3.00 g, 4.5 mmol) was swelled in DCM. 4-Acetoxy-3-nitro-benzoic acid (2.53 g, 11.25 mmol) was dissolved in DMF (7.5 ml) and triethyl-amine (1.56 ml, 11.25 ml) mixed with the drained resin. The mixture was placed on a shaker for 18h at rt. Followed by a careful wash with DMF (3x 2 min) and methanol (3 x 2 min). The resin was treated with a solution of 2-phanyl-ethyl amine (2M, 10 ml) in dichloromethane for 1 h at rt. and for 3h at rt. then washed with dichloromethane (3 x 2 min) and dried. 36.1 mg resin was added 1% TFA in DCM 10 min filtered, added hexane and evaporated to give 4-hydroxy-3-nitro-benzoic acid (7.8 mg), which correspond to a loading of 1.18 mmol/g.
NMR ($CDCl_3$): δ 8.81 (d, 1H), 8.20 (dd, 1H), 7.19 (d, 1H).

General procedure for the synthesis of nitro phenol esters:

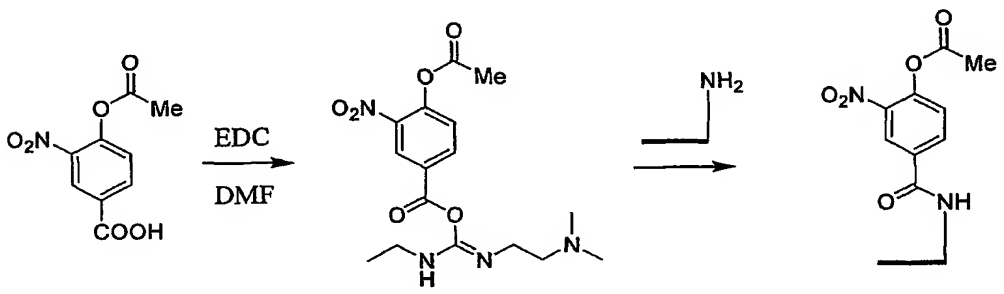
4-hydroxy-3-nitro-benzoic acid-(2-chloro-tritylresin) ester (0.173 g, 0.200 mmol) pre-swelled in DCM and drained, was subsequently added a solution of the appropriate acid (0.60 mmol, 3eq.) mixed with PyBrop (0.28 g, 0.60 mmol, 3 eq.) in DMF (0.5 ml), triethylamine (185 µL, 1.32 mmol, 2.2 x 3 eq.) and DMF (0.25 ml). The resin was allowed to react for 18 h at rt. Washed carefully with DMF 3 x 2 min, DCM 3x 2 min.

Cleavage from the resin was done with 1 % TFA in DCM 2 x 1 ml for 10 min. The cleavage mixture was mixed with Hexane 5-10 vol/vol in order to remove the TFA by co distillation.

The nitro phenol ester was purified by normal phase HPLC 20% EtOAc in heptane (0.5 % AcOH) → EtOAc (0.5 % AcOH).

Structures and yields are given in figure 37.

Loading of functional entities on to oligonucleotides for form CCPN's carrying functional entities.



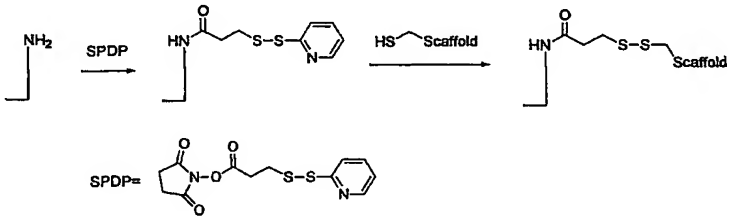
Synthesis of AH392/000247. 25 µl of 4-Acetoxy-3-nitro-benzoic acid (150 mM in DMF) was mixed with 25 µl EDC (150 mM in DMF) and the mixture was shaken for 30 min at 25°C. The mixture was added to 50 µl oligo AH392 (5-10 nmol) in 100 mM HEPES pH 7.5 and incubated with shaking for 20 min at 25°C. Excess building block was removed by extraction with EtOAc (500 µl) followed by two spin column filtrations and analysed by ES-MS and functional transfer assays (data not shown).

Synthesis of other loaded oligonucleotides. Organic fragments shown in Figure 37 are all loaded on the AH393 and AH394 oligonucleotides, to give the corresponding loaded oligonucleotides AH393/000138, AH394/000138 AH393/000387, etc., using a similar protocol.

Synthesis of AH381/scaffold. A hexameric scaffold peptide with the sequence Cys-PhePheLysLysLys was synthesised by standard solid-phase Fmoc peptide chemistry. The scaffold peptide comprises a –SH group on the cysteine side chain, said –

SH group being used for coupling the scaffold peptide to an amine-bearing oligonucleotide, whereby an anchor CCPN/scaffold like CCPN is formed. Each of the three lysine moieties comprises an amino group in the side chain. The amine groups are used as functional entity reactive groups for the formation of a connection to functional entities emanating from substitutent like CCPN's.

The N- and C-terminus of the peptide is capped to avoid any participation in the reactions to follow and subsequently purified by reverse phase-HPLC. The scaffold peptide is covalently attached to DNA oligonucleotide using the scheme shown schematically below. For illustrative purposes, the scaffold is indicated as HS Scaffold.



5 nmol of oligonucleotide AH381 in 100 mM Hepes-OH pH 7.5 is incubated with 20 mM Succinimidyl-propyl-2-dithiopyridyl (SPDP, Molecular probes) dissolved in DMSO for 3 hours at 25 °C. Excess SPDP is removed by triple extraction using 5 volumes of ethylacetate. The sample is further purified using a Bio-rad Microspin 6 column equilibrated in H_2O. 1 µmol hexapeptide is mixed with 5 nmol SPDP activated oligonucleotide in 100 mM Hepes-OH pH 7.5 for 2 hours at 25 °C. Excess peptide is removed by double sodium-acetate/ethanol precipitation of the scaffold-DNA complex according to standard procedure. The synthesis of AH381/scaffold is finally verified by Electrospray Mass Spectrometry (ES-MS).

Synthesis of small molecule (hexapeptide where the sidechain of the two lysines have been acetylated): Mix 10 µl buffer A with 1 pmol CCPN 0 (AH381/scaffold), 2 pmol CPN T1 (AH379) and 3 pmol CCPN T2 (AH294), 4 pmol CPN T3 (AH380), 5 pmol CCPN 2 (AH393/000247), and add H_2O to 50 µL. Anneal from 80° C to 20° C (-1°C/min.). Optionally dilute 100-fold. Incubate at 10°C for 5 sec. and then 35°C for 1 sec. Repeat 10-35° cycling o/n. If the sample was diluted 100-fold above, the

sample is now concentrated 100-fold by e.g. ethanol precipitation, filtration or like procedures. Add 5 pmol CCPN 3 (AH394/000247). Anneal from 80° C to 20° C (-1°C/min.). Optionally dilute 100-fold. Incubate at 10°C for 5 sec. and then 35°C for 1 sec. Repeat o/n.

The synthesis of the small molecule is verified by mass spectrometry, ELISA, Western blotting or other means of characterization. Optionally, the small molecule or the small molecule attached to CCPN0 (AH381) is purified before its analysis. Alternatively, the small molecule may be synthesized in large scale by performing the above reactions in 100 fold higher volumes and 100 fold larger amounts of material. The synthesis of the desired molecule may be verified by ELISA assays (using antibodies raised against the small molecule), or by mass spectrometry or other means.

Other small molecules, employing the hexapeptide as scaffold and the organic fragments of Figure 37 as substituents, can be made by combining the appropriate CCPN2 and CCPN3 oligonucleotides (carrying the desired organic fragments) with the CCPN0 (AH381/scaffold) oligonucleotide, and performing the above protocol. Again, the small molecules synthesized may be analysed by mass spectrometry, ELISA, and like methods, as described above.

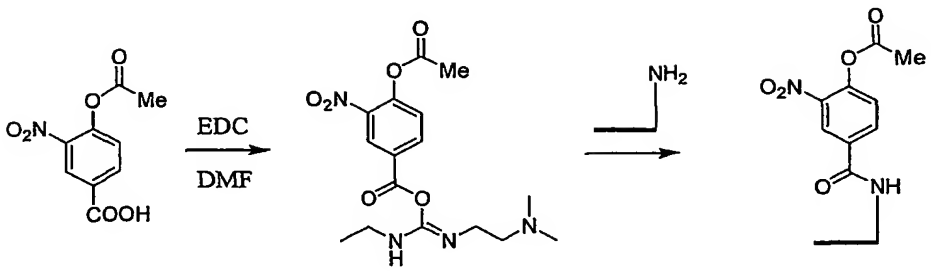
Example 2H: Synthesis of a small molecule through the reaction of functional entity reactive groups on four CCPN's.

In this example the set-up described in figure 30 is employed to synthesize a small molecule, where four chemical moieties are combined by the CPNs and CCPNs. This is also an example of the oligonucleotide complex depicted in "figure 4, claim 2 (see also figure 36 for explanation). Finally, this is also an example of circular structures such as depicted in "Figure 4, claim 1, 7-8, and 10-11.

Experimental.

Synthesis of functional entities as described in example 2G.

Loading of functional entities on oligonucleotides.



Synthesis of AH392/000247. 25 µl of 4-Acetoxy-3-nitro-benzoic acid (150 mM in DMF) was mixed with 25 µl EDC (150 mM in DMF) and the mixture was shaken for 30 min at 25°C. The mixture was added to 50 µl oligo AH392 (5-10 nmol) in 100 mM HEPES pH 7.5 and incubated with shaking for 20 min at 25°C. Excess building block was removed by extraction with EtOAc (500 µl) followed by two spin column filtrations and analysed by ES-MS and functional transfer assays (data not shown).

Synthesis of other loaded oligonucleotides. Organic fragments shown in Figure 37 are all loaded on the AH392, AH393, and AH394 oligonucleotides, to give the corresponding loaded oligonucleotides AH392/000138, AH393/000138, AH394/000138, AH392/000387, etc., using a similar protocol.

Synthesis of AH381/scaffold. See example 2G.

Synthesis of small molecule (hexapeptide where the sidechain of the three lysines have been acetylated): Mix 10 µl buffer A with 1 pmol CCPN 0 (AH381/scaffold), 2 pmol CPN T1 (AH379) and 5 pmol CCPN 1 (AH392/000247), and add H_2O to 50 µl. Anneal from 80° C to 20° C (-1°C/min.). Optionally, dilute 100-fold. Incubate at 10°C for 5 sec., 35°C for 1 sec. Repeat o/n. If the sample was diluted 100-fold above, the sample is now concentrated 100-fold by e.g. ethanol precipitation, filtration or like procedures. Add 3 pmol CCPN T2 (AH294), 4 pmol CPN T3 (AH380) and 5 pmol CCPN 2 (AH393/000247).
Anneal from 80° C to 20° C (-1°C/min.). Optionally dilute 100-fold. Incubate at 10°C for 5 sec. and then 35°C for 1 sec. Repeat o/n. If the sample was diluted 100-fold above, the sample is now concentrated 100-fold by e.g. ethanol precipitation, filtra-

tion or like procedures. Add 5 pmol CCPN 3 (AH394/000247). Anneal from 80° C to 20° C (-1°C/min.). Optionally dilute 100-fold.
Incubate at 10°C for 5 sec. and then 35°C for 1 sec. Repeat o/n.
The synthesis of the small molecule is verified by mass spectrometry, ELISA, Western blotting or other means of characterization. Optionally, the small molecule or the small molecule attached to CCPN0 (AH381) is purified before its analysis.
Alternatively, the small molecule may be synthesized in large scale by performing the above reactions in 100 fold higher volumes and 100 fold larger amounts of material. The synthesis of the desired molecule may be verified by ELISA assays (using antibodies raised against the small molecule), or by mass spectrometry or other means.

Other small molecules, employing the hexapeptide as scaffold and the organic fragments of figure 37 as substituents, can be made by combining the appropriate CCPN1, CCPN2 and CCPN3 oligonucleotides (carrying the desired organic fragments) with the CCPN0 (AH381/scaffold) oligonucleotide, and performing the above protocol. Again, the small molecules synthesized may be analysed by mass spectrometry, ELISA, and like methods, as described above.

Example 2I: Synthesis of a library of small molecules, each comprising three (functional entities).

In this example the set-up described in figure 30 is employed to synthesize a library of small molecules.

Experimental.

Synthesis of functional entities.

The ten nitro phenol esters shown in figure 37 are synthesized as described in example 2G. The ten nitro phenol esters are loaded on specific oligonucleotides, i.e. a specific nitro phenol ester is loaded on a specific oligonucleotide sequence. Two sets of oligos are used, namely CCPN 2 and CCPN 3 oligos (DNA oligos that anneal to adjacent positions on CPN 3T). Ten CCPN2 and ten CCPN3 oligo sequences are loaded with the ten nitro phenol esters. In other words, a total of

twenty loaded oligos are generated. In addition, the CCPN 0 oligo (AH381/scaffold) , described in example 2G, is synthesized. Finally, the sequences of CPN T1, CPN T2 and CPN T3 are designed in a way so that these oligos anneal to each other and to CCPN0, CCPN2 and CCPN3 as indicated in Figure 30.

Synthesis of a 100-membered small molecule library (hexapeptides where the side chain of the two lysines have been acylated with the various chemical moieties from the nitro phenol esters): Mix 10 µl buffer A with 1 pmol CCPN 0 (AH381/scaffold) oligo, 2 pmol of each of the CPN T1 oligos and 3 pmol of each of the CCPN T2 oligos, 4 pmol of each of the CPN T3 oligos, 5 pmol of each of the CCPN 2 oligos, and add H_2O to 50 µL. Anneal from 80° C to 20° C (-1°C/min.). Optionally dilute 100-fold. Incubate at 10°C for 5 sec. and then 35°C for 1 sec. Repeat 10-35° cycling o/n. If the sample was diluted 100-fold above, the sample is now concentrated 100-fold by e.g. ethanol precipitation, filtration or like procedures. Add 5 pmol of each of the CCPN 3 oligos. Anneal from 80° C to 20° C (-1°C/min.). Optionally dilute 100-fold. Incubate at 10°C for 5 sec. and then 35°C for 1 sec. Repeat o/n.
After synthesis of the library, the library molecules (DNA-small molecule complexes) may be purified by e.g. ethanol precipitation or by other means. Then molecules with a given characteristic may be isolated from the library, for example by performing an affinity chromatography selection, and the isolated molecules can then be identified by amplifying the recovered DNA molecules and sequencing of these. Alternatively, the small molecule library may be synthesized in large scale by performing the above reactions in 100 fold higher volumes and 100 fold larger amounts of material. The selection of molecules with desired characteristics may be done by immobilization of a target protein onto the sides of a reagent tube, and exposing the library to this coated surface; or by incubating the library with a protein target in solution, followed by immuno precipitation to isolate the ligands of the target protein; or by incubating the library with a protein target in solution, followed by gel mobility shift assays to isolate the ligands of the target protein; etc.